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ON SOME PLANT PARASITIC NEMAS AND RELATED FORMS[1]

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A CEPHALOBUS LIVING IN THE GREEN LEAVES OF PHLOX DRUMMONDII

PREVIOUS FINDINGS

Few *Cephalobi* have been mentioned as infesting plants. De Man (see Ormerod *13*),[2] found oats appearing as if infested by *Tylenchus dipsaci* Kühn, to harbor instead many *Cephalobus rigidus* (A Schneider). Ritzema-Bos (*15 p. 327*) observed in *Allium proliferum* infested with *Tylenchus dipsaci* and other nemas, specimens of *Cephalobus rigidus* and *elongatus* de Man. In 1891 (*16*) he again found two *Cephalobus* species in the healthy, as well as in the diseased, parts of strawberry plants suffering from cauliflower disease, caused by *Aphelenchus ormerodis*, and considered one species closely related to, or identical with, *C. nanus* de Man, and the other with *C. rigidus*. In some plants the latter was more numerous than the *Aphelenchus;* but since all contained *Aphelenchus*, and only a majority *Cephalobus*, the origin of the disease was attributed to the former. In the first important study of a plant-attacking *Cephalobus*, 1906 and 1909, Kati Marcinowski (*8, 10*) showed by many experiments that *C. elongatus* enters healthy plants (rye, etc.) and, if numerous, may kill, or at least injure, the host plant.

NEW OBSERVATIONS

August, 1923, Florence Hedges submitted mature Phlox plants from Quaker Neck, Chestertown, Md. The plants were green with only a few brown spots, but dwarfed, less bushy, and with fewer flowers than in previous years. Larval and adult specimens of *Cephalobus* were fairly numerous within the green leaves and stems of these plants, but not massed. When the leaves were covered with water, many nemas soon left them and sank by hundreds to the bottom.

The *Cephalobus* proved to be *C. subelongatus* Cobb (*3*), a species based on a single female. Dr. N. A. Cobb placed at the writer's disposal a sketch of a male tail end, and notes in which this species is mentioned as living on green pepper pods, Philippine Islands, on clover seeds, Idaho, and on lily of the valley roots (*Convallaria majalis*), Washington, D. C. He has also observed *C. subelongatus* on diseased germinating rubber seed (Castilloa) in Hawaii, and in crowns of diseased alfalfa plants from New Jersey and Pennsylvania.

[1] Received for publication Jan. 28, 1924—issued November, 1924.

[2] Reference is made by number (italic) to "Literature cited," pp. 1065–1066.

TECHNICAL DESCRIPTION

Supplementing Cobb's description: The two lateral wings may be rather well developed or more or less lacking. Each of the three main lips (one dorsal and two ventro-submedial) separated from each other by a rather deep incision (Pl. 1 B), is again divided by a shallow concavity into two points, each with a papilla (Pl. 1, A and C). The two lateral points of the ventro-submedial lips, somewhat smaller than the others, apparently bear the amphids. They are very difficult to see, open behind the lateral lips, and are slightly shifted dorsad (Pl. 1, Aa and C and Pl. 4, E and F). The exceedingly narrow opening is circular or oval-shaped, and leads into a long, somewhat conical cavity (amphidial pouch). Within the latter a small number of fine fibers, possibly six, can be seen; they show spindle-shaped swellings (Pl. 4, Ca and D) and are very similar to the elements in the chemical sense organs of other animals (organs of taste as well as organs of smell). It may, therefore, be quite safe to regard these amphids as the chemical sense organs of the present species until a better or more exact explanation can be given. The figures (Pl. 4, C, D, E, and F) make any longer description needless. A front view shows six papillae; the lateral are slightly smaller (Pl. 1, E). The mouth cavity (Pl. 1, A and E) agrees with Cobb's data, but it differs from sketches of *Cephalobus elongatus* by De Man and Marcinowski.

The cardiac valvular apparatus (Pl. 1, D) consists of three groups of cutinized parts; the valves have the usual ribbed structure (Pl. 1, D); two other groups of three elements each occur farther back (Pl. 1, D, valves 2 and 3). These drawings are from live specimens. Any given circumference of the intestine comprises only two cells (Pl. 1, F).

Examination of over 30 specimens showed the excretory pore usually ventrad of the middle of the cardiac bulb, seldom fore or aft; the alimentary tube being capable of forward and backward movement, the relative position of pore and bulb changes somewhat even in the same specimen. The well developed outlet leading to the excretory pore is connected with at least one large and two or three smaller cells ventrad of the beginning of the oesophagus (renette cells or ventral glands).

Cobb described the female sexual apparatus. Plate 2, A, shows the part of the uterus which serves as the *receptaculum seminis* with the sperms in an interesting arrangement. Each relatively large sperm consists of a transparent part, well separated from a straight-edged, granulated part, and along this edge the rod-like granules are all similarly placed. In many other nemas the spermatozoa take on a definite orientation within the receptaculum, as if influenced by some tropism; the head is then directed towards the outlet of the oviduct and the sperms are massed together as if competing for the place nearest the arriving egg. Here no such condition of the sperms can be seen; as Plate 2, A shows, the clear transparent part of the spermatozoa, perhaps corresponding to the "glanzkörper" of the Ascaris sperms, may be orientated any way. It may be added that these sperms are capable of amoeboid movements. Plate 4, B, a–i, are sketches made of such moving sperms; the pseudopodial processes are only finely granulated, the larger granules resting behind; they then show no such definite arrangement as sketched in Plate 2, A.

No description of the male has been published. The sexual number is 53.1, that is, to every 100 females there are 53.1 males. (A total number of 170 specimens was examined with regard to sex.) In the single, reflexed, male sexual organ, *vesicula seminalis* and *ductus ejaculatorius* are not well set off from each other. Plate 1, G and H illustrate the curved, paired and symmetrical spicula, each with a strengthening rib and a somewhat cephalated proximal end. A

ledge which soon tapers and nearly disappears springs from the cephalated part and perhaps forms either the edge of a membranous, wing-like, lateral part of the spicula, or the cutinized part where the very inconspicuous and weakly developed *protractores spiculorum* are attached (Pl. 1, N). The copulatory muscles are indistinct and the details of the bursal muscles doubtful, a result of the small number of muscular fibrils, rather than absence of the muscles. As to the papillae typical of male Cephalobi, the present material shows much variation in number and location (Pl. 1, I, J, K, L, and M). The European *Cephalobus elongatus* De Man is, as Cobb remarked, perhaps identical with the present form; but it differs in the pharynx [see descriptions by De Man (*7*) and Marcinowski (*8*)] in the position of the papillae of the male; and also the position of the excretory pore is much farther cephalad. De Man (*7*) shows the gubernaculum of *elongatus* linear, states its great variability, but omits morphological details. Thus the relationship of the two forms is uncertain, but the variability, with transgressional characters of both, suggests identity. The following formulae indicate much variation in the length of the individuals and of the organs:

female $\frac{1.4 \quad 14. \quad 21. \quad '58. \quad 93.4}{1.3 \quad 3.1 \quad 3.7 \quad 4.6 \quad 2.5}$ 0.935 (0.7–1.4)mm. (11 specimens measured).

male $\frac{1.1 \quad 15. \quad 20. \quad 'M \quad 94.1}{1.3 \quad 3.1 \quad 3.3 \quad 3.5 \quad 2.7}$ 0.863 (0.74–1.08)mm. (9 specimens measured).

Females		Males	
α–21.9 (19.6 –29.4)	n–11.	α–28.0 (27.0–29.4)	n–9.
β– 4.8 (4.34– 5.88)		β– 4.9 (4.6– 5.1)	
ψ–15.1 (11.8 –25.0)		ψ–11.2 (14.9–20.0)	

The largest specimens were from cultures on concave slides in a drop of water containing parts of grains, leaves, and stalks of corn (Zea mays); various other plant materials were also used with success.

Cobb succeeded in rearing *subelongatus* in macerated silage; in this case cockroach meat was also used, both foods being productive of good results. *Subelongatus* may therefore be regarded as omnivorous and well fitted for varied life conditions. More attention should be given to the ability of the genus *Cephalobus* to enter and live in healthy plants; hitherto the inclination has been to disregard Cephalobi as the cause of injury to plants.

KIGELIA PINNATA, THE AFRICAN SAUSAGE TREE, AS A HOST PLANT OF HETERODERA RADICICOLA

A knowledge of plants subject to root-knot, caused by *H. radicicola*, is of importance to horticulture, agriculture, and forestry.

Kigelia pinnata, the sausage tree, must be added to the list of host plants of *H. radicicola*, since young plants, apparently soil-infested, raised from seed and brought by Mr. Woods of the inspection office of the Federal Horticultural Board, showed attacks of this parasite. It may also be noted that snapdragon (*Anthirrhinum majus*) plants from Washington, D. C., were badly infested by *H. radicicola*.

REMARKS ON THE CHRYSANTHEMUM NEMA, APHELENCHUS RITZEMA-BOSI SCHWARTZ, AND ITS PRESENCE IN THE UNITED STATES

The presence of this nema pest in the United States has not yet been definitely established. Atkinson (*1*), mentioned an *Aphelenchus* sp. causing disease in chrysanthemum buds from Baltimore, Md.

In August, 1923, chrysanthemum and *Phlox drummondii* from Washington, submitted by Florence Hedges, were found infested by *Aphelenchus ritzema-bosi*, as were also chrysanthemums sent later from Quaker Neck, Chestertown, Md. The chrysanthemums showed lesions similar to those mentioned by Osterwalder (*14*), that is, the leaves partly yellow, or yellow and brown. The phlox leaves were mostly dried or yellow, very few being green. Nemas were numerous in the chrysanthemum leaves, but few in the phlox, where they were found in the leaf blades and stem. Although there are Aphelenchi said to infest different plants, some closely related to, some synonymous with *ritzema-bosi*, comparative examination of many specimens from all these host plants is necessary before a final conclusion is reached. Opinion on the views of Ritzema-Bos (*17*), Marcinowski (*9*), Molz (*12*), Schwartz (*19*), Stewart (*21*) and Goodey (*6*) on the taxonomic position of these nemas is, therefore, withheld.

The species here mentioned most closely resembles *A. ritzema-bosi* Schwartz, hitherto known only from chrysanthemums. As Goodey recently stated (*6*), this form is undoubtedly synonymous with Stewart's *A. phyllophagus;* also the specimens from South Africa mentioned by Sandground (*18*) as *A. phyllophagus* Stewart, belong to *A. ritzema-bosi.*

In August, 1923, Dr. Sandground sent the writer *A. ritzema-bosi* in dried chrysanthemum leaves kept since February, 1921. They were partly revived in water. *Only larval specimens of the stage before the last moult revived*, the first specimens reviving after 18 hours and soon moulting. The present species, therefore, can stand desiccation for at least 22 months. When again dried for from 2 to 5 weeks, they revived once more *in a much shorter time*—10 to 12 hours. Specimens which moulted after 22 months of dormancy withstood afterwards a drying of 3 weeks in the adult stage.

The specimens from Washington and Chestertown had fine annules and two sublateral wings on each side. The head seen laterally was similar to that drawn by Schwartz (*19, p. 312, fig. 12*). The excretory pore, on which Stewart lays much stress (*21*), was rather variable in position; in some specimens scarcely the length of the oesophageal bulb behind this latter and rather in front of the nerve-ring; in others, well behind the nerve-ring. The male tail end resembles that figured and described by Schwartz (*19*) and Stewart (*21*) for *A. ritzema-bosi*, except some specimens that show a papilla near the middle of the ventral side and just in front of the very end (Pl. 2, B). In other specimens this papilla was scarcely seen or not at all; in which case no true papilla was formed, but only a fine tube. It is uncertain whether this was the outlet of a gland, no glandular cells being seen to be connected with it. Therefore, it is believed that the present species has really two ventro-median postanal papillae in the male, the most inconspicuous one being near the tail end. In some specimens the papillae are easily seen with only a low magnification, and at first the writer thought it to be *A. helophilus* de Man as figured by *Kati Marcinowski* (*9*). Plate 2, B shows a lateral view of such a male tail end. The writer has no additional information as to the effect on the host. The observation of former investigators that the nemas leave the plants when put in water can be confirmed.

REMARKS ON DORYLAIMUS REGIUS DE MAN AND ITS FOOD

Rather large-sized species of the nema family Dorylaimidae are numerous in most soils; hence the question of their economic significance and relationship arises. On account of the spear-like onchium and sucking apparatus, a more or less parasitic relationship to plants has been pointed out by investigators. In common with various other nematologists, the writer has observed Dorylaimi with green intestinal contents, in certain cases recognizable as chlorophyll.

Thus, the belief that some Dorylaimidae feed partly on plants seems well founded; however, this is apparently not true for all species. It is doubtful whether the Dorylaimus species, so numerous at great depths in fresh-water lakes, live on plants; as plants seem to be unavailable there, a carnivorous feeding habit is more probable. Many soil-inhabiting species of Dorylaimus and related genera also may be carnivorous.

The following study of the intestinal contents of *Dorylaimus regius* de Man indicates that at least this species seems to be carnivorous. September, 1923, six *Dorylaimus regius* De Man (four females; two males) were submitted by the Plant Quarantine Inspection Service for identification. Four, found by R. G. Cogswell, of Philadelphia, Pa., were about roots of Amaryllis imported from Germany, and two about roots of roses from Norway. A study of these enables the writer to add to the knowledge of this rather uncommon species. Measurements, Cobb's formula:

$$\text{female } \frac{0.\quad 6.1\quad 15.\quad 12.5\quad 11.0}{0.5\quad 1.5\quad 1.6\quad 2.1\quad 1.5} \quad '51'.\ 99.0 \quad 6.919 \text{ mm.}$$

$$\text{male } \frac{0.\quad ?\quad 17.1\quad -M-}{0.5\quad ?\quad 1.9\quad 2.1} \quad 98.6 \quad 5.848 \text{ mm.}$$

De Man's formula:

Female+1	Female+2	Male	
α—40. 5	47. 9	47. 1	number of preanal male.
β— 6. 18	6. 4	5. 7	papillae in both cases, +17.
ψ—93. 5	100. 7	86. 0	plus two anal papillae.
v—52. 3%	51. 3%		

The two Norwegian specimens (females) differed chiefly in the length of the tail, which was much shorter and showed measurements as follows:

Female 1		Female 2	
Total length —	5. 686 mm.	Total length	7. 820mm.
α—	38. 0		51. 4
β—	6. 08		6. 2
ψ—	133. 8		154. 3
v—	50. 5		47. 0

The typical *Dorylaimus regius* De Man had hitherto been found only by De Man (*7*), Brakenhoff (*2*), and Marcinowski (*8*). Micoletzky (*11*) considers *D. superbus* de Man to be a small variety. Perhaps also *D. eurydoris* Ditlevsen belongs to *D. regius* (*5*).

The cuticle has at least three layers (Pl. 3, F); the surface layer shows a transverse striation similar to the lines of a finger-print (Pl. 3, G), and sometimes a faint indication of cross fibers, as in many Mermithids. The number of cephalic papillae seems greater than hitherto described; the lateral lips have only two papillae, but each submedial lip has three (Pl. 2, C, D, E, F, and G). The papillae of the second circlet are double on the submedial lips. Another organ hitherto not mentioned for the present form is the amphid (lateral organ). The whole lip region apparently can be protruded or retracted, and the amphids are accordingly seen more or less well. In Plate 2, E, the lip region is somewhat protruded and the amphid is partly exserted; whereas in Plate 2, C and D the whole organ, hidden inside the ridge just behind the lip region is disclosed as a broad pouch-like cavity with a great number of bent and curved terminals (Endfasern) as in a few other Dorylaimidae studied. The wall of this pouch-like structure is thicker in the specimen in Plate 2, C and D than in that of E. As Plate 2, F shows, the pouch is somewhat divided into an anterior and a posterior part, and at its base enters what the writer regards as the "amphidial" nerve with its numerous fibers, surrounded by a large cell of apparently glandular character, which can be followed a distance towards the nerve-ring. Plate 2, G shows the amphids of the specimens of Plate 2, C and D, in a front view.

As described by Brakenhoff (*2*), papillae more numerous ventrally than dorsally, can be observed along the entire body in the lateral and submedial regions. The writer believes them to be rather of nerve character than outlets of glands as Brakenhoff suggests (Pl. 4, A; Pl. 2, C and D; and Pl. 3, A, B, C, and D). There seems to be a rudimentary excretory pore ventrad from the nerve-ring (Pl. 4, A), and several glandular cells at the cardia, but apparently all on the dorsal side of the oesophagus and intestine.

Plate 3, C and D show that the specimens from Germany (Pl. 3, C) had a longer, less blunt tail than those from Norway. In both males and females, papillae similar to the body papillae could be seen on the tail end, their position differing in each specimen. At the very end of the tail the cuticle showed in one specimen (Pl. 3, A and B) a tenon-like element of somewhat darker color, not quite reaching the terminal surface. Another had the same region radially striated (Pl. 3, C), the rest of the animals showing none of these structures. Therefore, these and similar features, as they occur in other Dorylaimus species, are probably of little taxonomic value.

Since only a single male has so far been found, or possibly two (if Ditlevsen's *D. eurydoris* belongs to the present species), some information regarding it may be added. The spicula are sketched in Plate 3, A and B. There are two gubernacula; what probably are details of their actuating muscles are also figured. Most interesting are the papillae of the male, which differ in the two present specimens (Pl. 3, B and L). In one specimen the series of preanal, contiguous papillae, beginning somewhat in front of the spicula was regular and continuous (Pl. 3, B), while in the other specimen the posterior papillae of the series were separated from the others by a long interval. The first specimen in all respects resembles the male *D. eurydoris* as described by Ditlevsen (*5*). Brakenhoff's male (*2*) has two additional papillae and the series of papillae begins directly opposite the proximal ends of the spicula.

ECONOMIC SIGNIFICANCE AND FOOD OF DORYLAIMUS REGIUS

In estimating the economic significance of soil nemas, a knowledge of their food and feeding habits, of which very little is known, is of chief importance. There are two ways to determine the food of these nemas, (1) by experimental feeding, (2) by a study of the intestinal contents. The first is difficult on account of the environment—the soil; the second, because the sucking way in which most forms feed renders the intestinal contents more or less unrecognizable; nevertheless, knowledge important to the study of soil economy can be gained by careful study of the intestinal contents. Such a study of the four specimens of *D. regius* resulted in finding an oligochaete seta (Pl. 3, J); there is figured at the right of the bristle, at the same magnification, the spear of the *Dorylaimus*, to show that the entrance of the bristle through the spear was possible. In another specimen the intestine was filled with a gray mass containing many nuclei of such a nature that hardly anything other than the gonad of an animal can be considered the source of it. Therefore, *D. regius* undoubtedly ingests animal matter. In an earlier paper Steiner (*20*) called attention to the apparent relationship of the Mermithidae to the Dorylaimidae, these latter being postulated as the ancestors of the former. The fact that certain Dorylaimidae actually feed on animal food is clearly in harmony with this view.

PARATYLENCHUS NANUS COBB INFESTING THE ROOTS OF ZINNIA ELEGANS

Paratylenchus nanus Cobb (*4*) is perhaps synonymous with *P. bukowiensis* Micoletzky (*11, p. 606*). Cobb found his specimens about the roots of grass, Devil's Lake, N. Dak., and at Four Mile Run, Falls Church, Va. Micoletzky

had a single specimen from roots of grasses from a sandy meadow in the inundation of the Pruth River, near Czernowitz, Bukowina.

The present specimens were taken from the exterior tissues of roots of *Zinnia elegans* sent by Mary C. Brooks, Quaker Neck, Chestertown. The nemas, numerous where the surface of the root was brownish, occurred as single isolated specimens spread through the tissues. Although more than 200 specimens were closely examined, no males were found. The writer can, however, confirm the statement of Cobb that spermatozoa are present in the female uterus and that therefore the species must be considered syngonic. The roots showed no abnormalities other than the above-mentioned brownish surface spots, no swellings, etc., indicating that plants react very differently to various parasitic nemas. This seems to the writer to be additional evidence that the chief damage Heterodera, some Tylenchi and Aphelenchi do to their plant hosts is caused by the secretion of toxic nemic substances. Perhaps different plant species, and even different varieties of the same species, vary greatly in their susceptibility to these toxins, and consequently the size or number of the deformations of plants may not be an index to the number of parasitic nemas living therein.

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PLATE 1

A.—*Cephalobus subelongatus* Cobb: head end sublateral view; amph, amphid.

B.—Same; head end: m inc, main incision between main lips.

C.—Same; one main subventral lip with the two papillae separated by a flat incision; m lb, main lip; subv ppl, subventral papilla; lat ppl, lateral papilla behind the amphid.

D.—Same; cardiac region of the body; cls, cells which apparently belong to the excretory apparatus; nrv r, nerve ring; p ex, excretory pore; ren, renette or ventral gland; t p, transparent tissue of the cardiac region of the intestine; valv, the ribbed valvulae of the valvular apparatus; valv el 2, second group of three rod-like valvular elements; valv el 3, third group of three valvular elements of same form as those of group two.

E.—Same; front view of the head end; amph, amphid; sub p, submedial papilla; lat p, lateral papilla.

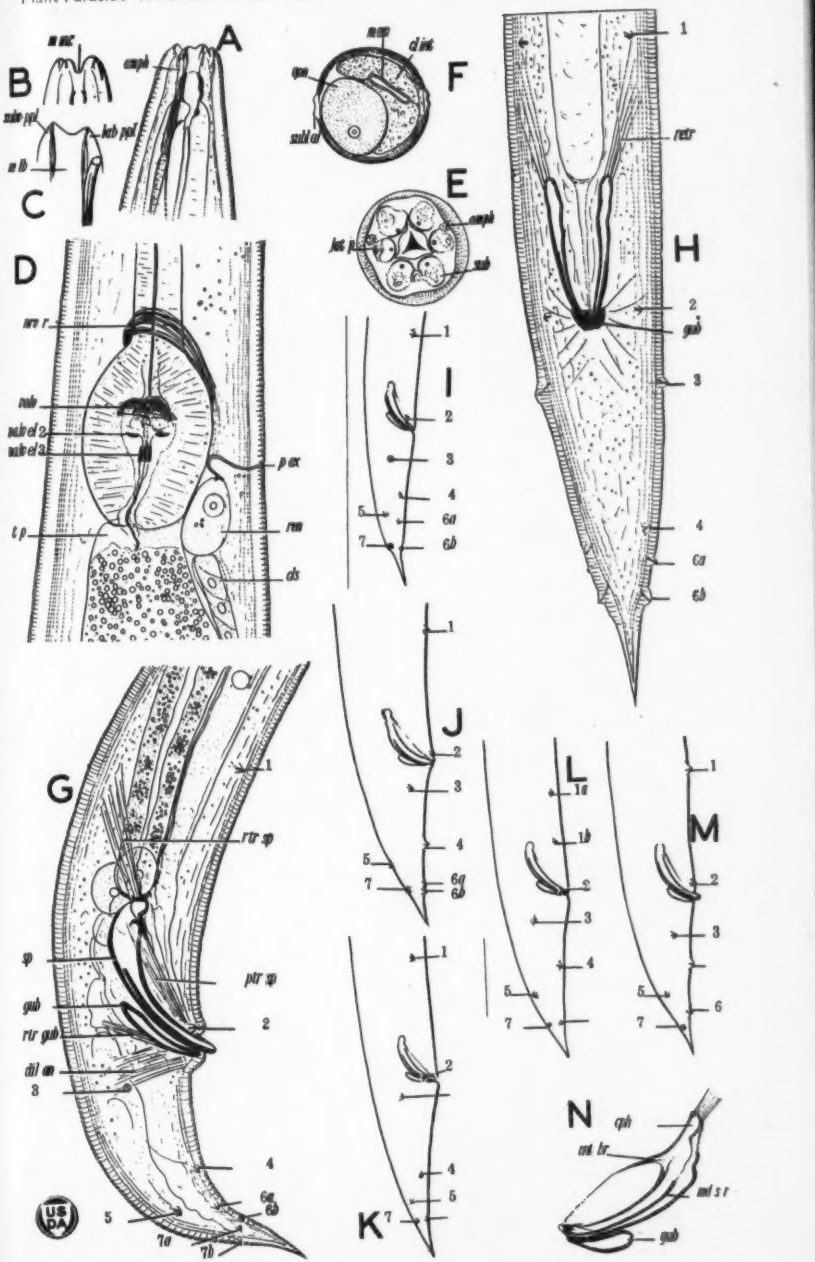
F.—Same; cross section through the middle region of the body; cl int, intestinal cell; mem, cutinized membrane around intestinal cavity; gon, gonad; subl al, sublateral wing.

G.—Same; tail end of a male; dil an, dilatator muscle of anus; gub, gubernaculum; rtr sp, retractor spiculi; ptr. sp, protractor spiculi; sp, spiculum; 1–7, papillae of the male tail end; some double, as 6a, 6b, 7a, 7b.

H.—Same; ventral view of the male tail end; gub, gubernaculum; retr, retractor spiculi; 1–6, papillae of the male tail end; number 6 is double, number 7 can not be seen as situated on the dorsal side.

I, J, K, L, and M.—Same; sketches of different male tail ends to show the variations in the number and position of the papillae, of which some are double.

N.—Same; a lateral view of the spiculum and the gubernaculum; cph, cephalated proximal end of spiculum; gub, gubernaculum; md s r, median strengthening rib of spiculum; vnt br, branch of the ventral rib.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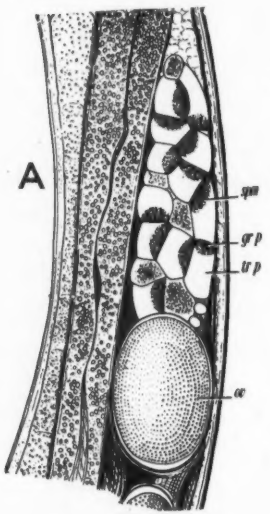
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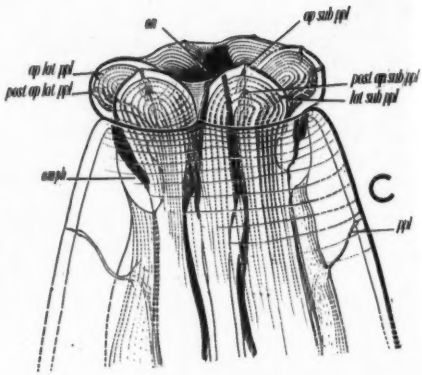
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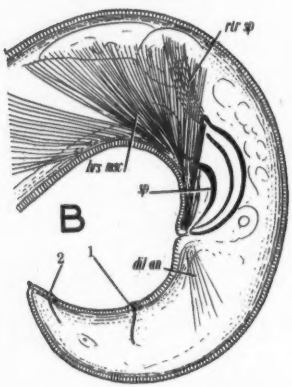
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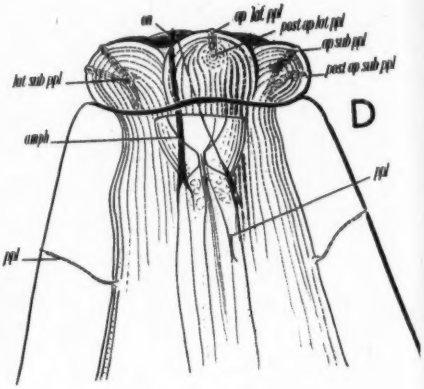
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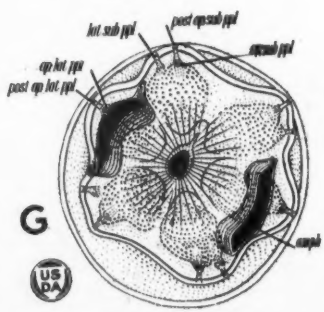
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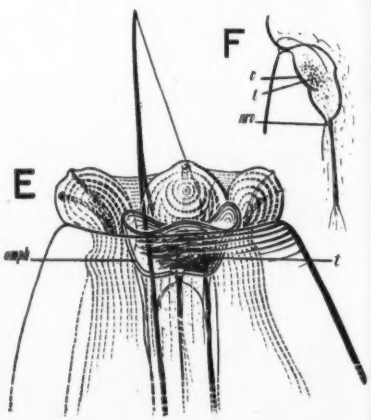
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PLATE 2

A.—*Cephalobus subelongatus* Cobb, region of female body with uterus filled with spermatozoa and eggs; gr p, granular part of a spermatozoón; ov, egg; trap, transparent part of a spermatozoón; spm, a spermatozoón.

B.—*Aphelenchus ritzema-bosi* Schwartz; tail end of a male in lateral view; brs msc, bursal muscle; dil an, dilatator muscle of anus; rtr sp, retractor muscle of spiculum; 1–2 papillae.

C.—*Dorylaimus regius* de Man; median view of the head end; amph, amphid; ap lat ppl, apical lateral papilla; ap sub ppl, apical submedian papilla; lat sub ppl, lateral submedian papilla; on, spear; post ap lat ppl, postapical lateral papilla; post ap sub ppl, postapical submedian papilla; ppl, papilla of the body surface.

D.—Same; lateral view of head end; lettering the same as in C.

E.—Same; lateral view of a head end with the opening of the amphid (amph) protruded and free; t, terminals.

F.—Same; optical section through the amphid in profile position; nrv, amphidial nerve; t, sections through terminals; v, amphidial wall.

G.—Same; front view of head end; lettering same as in C.

PLATE 3

A.—*Dorylaimus regius* de Man; ventral view of the tail end of a male; an ppl, anal papillae; d cut, differentiated cuticular tissue; gub, gubernaculum; ppl, ventromedian papillae; sp, spiculum; s ppl, skin papillae.

B.—Same; tail end of a male in lateral view; an ppl, anal papillae; brs msc, bursal muscles; d cut, differentiated cuticular tissue; gub, gubernaculum; ppl, series of preanal papillae; ptr sp, protractores spiculorum; rtr sp, retractor spiculi; 1–4 skin papillae.

C.—Same; tail end of a female from Germany; dil an, dilatator muscle of anus; ppl, skin papilla; str cut, radial striation of the cuticle.

D.—Same; tail end of a female from Norway; compare with C and note the much shorter tail.

E.—Same; transverse striation of the surface of the cuticle.

F.—Same; optical longitudinal section through the skin; hyp, hypodermis; 1–3 different layers of the cuticle.

G.—Same; one of the ventromedian preanal papillae.

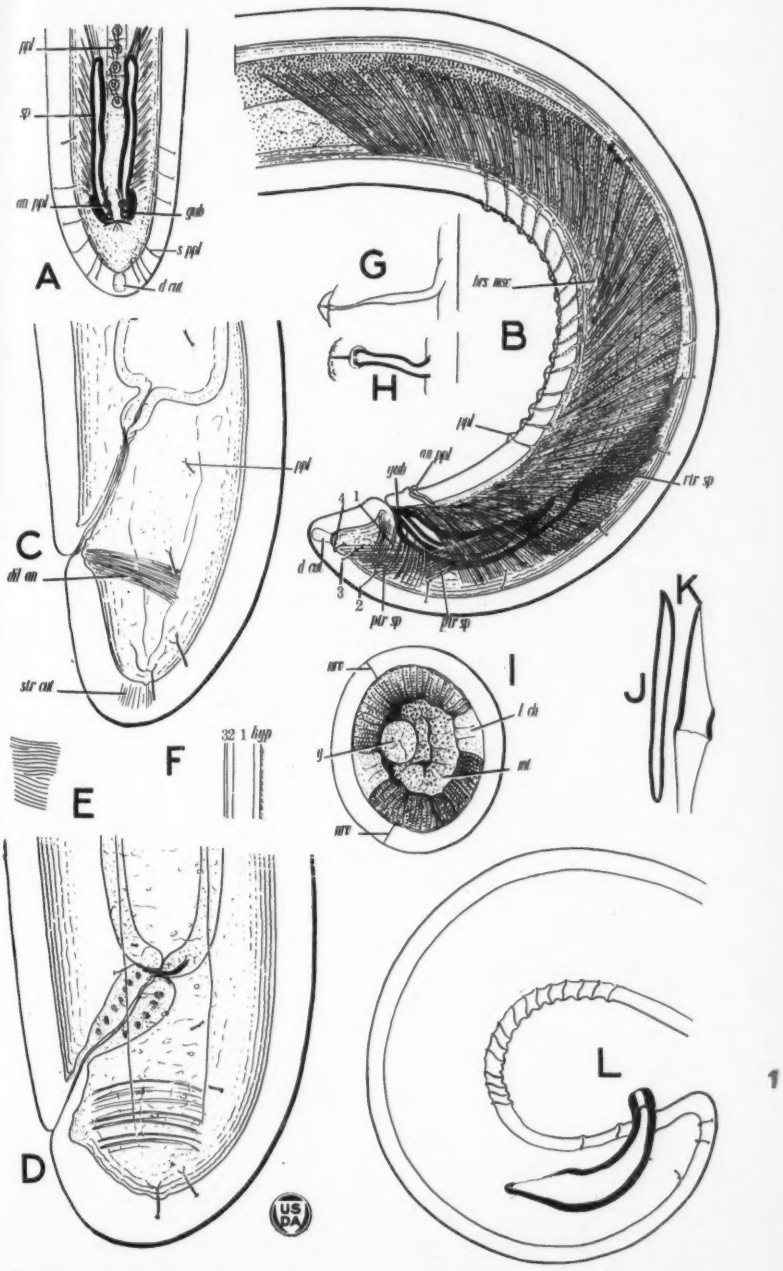
H.—Same; one of the anal papillae; compare the difference with G.

I.—Same; cross section through the middle region of the body; g, gonad; int, intestine; l ch, lateral chord; nrv, skin nerve.

J.—Sketch of a bristle of an oligochaete as found in the intestine of *Dorylaimus regius*.

K.—*Dorylaimus regius* de Man; the spear drawn with the same magnification as J to show the possibility of the entering of the bristle through its lumen.

L.—Same; tail end of a male with a different order of the ventromedian preanal papillae.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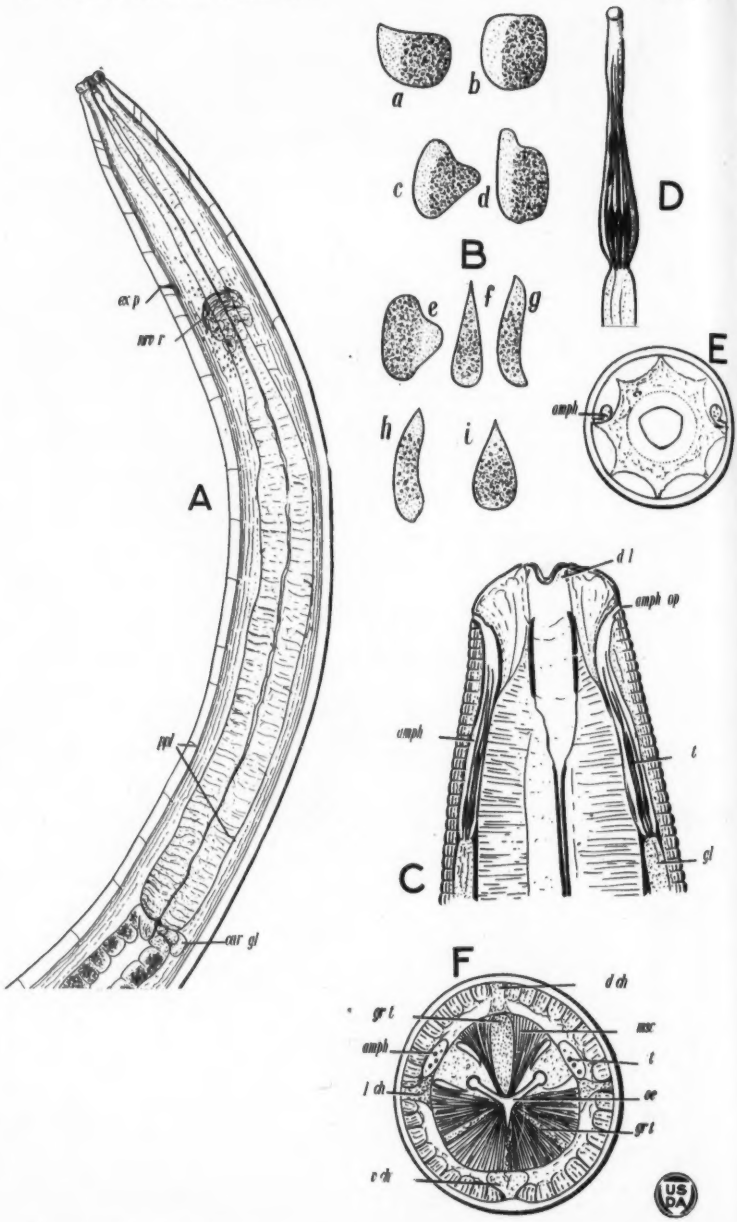
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PLATE 4

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PLATE 4

A.—*Dorylaimus regius* de Man; head end of the body with beginning of the intestine; car gl, cardiac gland; ex p, excretory pore; nrv r, nerve ring; ppl, skin papillae.

B.—*Cephalobus subelongatus* Cobb; a-i, sketches of spermatozoids showing amoeboid movements.

C.—Same; dorsal view of head end; amph op, amphidial opening; d l, dextero dorso submedial lip; gl, apparently glandular cell attached to the amphidial pouch; t, terminals (sensilles?).

D.—Same; amphid seen laterally.

E.—Same; optical cross section of the head just behind the lip region at the level of the amphidial openings; amph, amphid.

F.—Same; cross section behind the pharynx; amph, amphid; d ch, dorsal chord; gr t, granular tissue; l ch, lateral chord; msc, radial oesophageal muscles; oe, oesophageal channel; t, terminals, v ch, ventral chord.

oi

THE INFLUENCE OF ENVIRONMENT ON SEX IN HEMP, CANNABIS SATIVA L.[1]

By HUGH C. MCPHEE

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INTRODUCTION

Since environment apparently has such a potent influence on sexual expression in hemp, *Cannabis sativa* L., the writer found it necessary during the course of an investigation of the genetics of sex in this species, to study the effect of relative length of day and night upon vegetative and floral development. The problems connected with this phase of the work have been pursued only as far as was necessary to enable the major work to be carried on and the results are not to be considered as final from the physiological standpoint. But since the work has been discontinued it seems desirable to report the results that they may be available to others who are interested in problems of this kind.

Physiological studies similar to those reported here have been pursued by several investigators, especially Garner and Allard (*1*, *2*, *3*).[2] Since they have reviewed the literature which pertains to this phase of physiology, it seems unnecessary to attempt further review here except to mention Schaffner's work on hemp. Schaffner (*5*, *6*, *7*) found that apparently it is possible to control sex in hemp. When grown during the long days of summer the sexes remain pure and the ratio of staminate to carpellate plants is approximately 1: 1. When grown in the greenhouse during the short days of winter, however, both carpellate and staminate plants showed reversal to the opposite sex. Furthermore, the proportion of plants which showed sex reversal seemed to be roughly proportional to the length of the darkness period. In the shortest days of winter some of the plats showed almost 100 per cent of intersex plants. Schaffner therefore maintains that sex in hemp is non-Mendelian in nature and is under the control of environmental factors.

SEXUAL DIMORPHISM

When grown under normal conditions hemp is when mature dimorphic in both vegetative and flower characters. When grown in the greenhouse during the winter the dimorphism is even more pronounced. The principal sexual differences are as follows:

Staminate plant: More slender and taller than the carpellate plant because of the rapid elongation of the internodes just prior to anthesis; terminal inflorescences with practically no leaves; flowers normally with five sepals and as many anthers; much shorter life than the carpellate type (Pl. 1).

[1] Received for publication March 26, 1924—issued November, 1924. The results reported in this paper were obtained at the Laboratory of Plant Genetics, Bussey Institution of Harvard University, and completed in the U. S. Department of Agriculture.

[2] Reference is made by number (italic) to "Literature cited," p. 1080.

Carpellate plant: More vigorous but shorter than the staminate type; terminal inflorescence leafy; broad crown of leaves; flowers with perianth but no vestiges of stamens; weight at maturity about twice that of the staminate type; longer life.

Probably hemp is as dioecious as any of the so-called dioecious species but it is a well-known fact that certain individual plants sometimes bear both staminate and carpellate flowers at the same time, and that such intersex types appear in larger numbers when the daily exposure to light is short, such as prevails in northern latitudes during the winter months. The vegetative differences, however, are the same for the plants which remain sexually pure and for those which develop some flowers of the opposite sex. That is, a change in the sex of the flowers produced is not accompanied by a corresponding change in the vegetative characters. In other words, the dimorphic vegetative types occur in approximately a 1:1 ratio. It seems quite clear that probably the vegetative type of the plant was already determined in the embryo from which the plant grew but that the sex of each flower was not determined until a somewhat later stage. The natural thing is for each vegetative type to produce flowers of the sex characteristic of that type but so far as any differences which exist in the structural elements of the two types are concerned there is no reason why both sexes of flowers can not be borne by both staminate and carpellate plants. The common occurrence of intersex types in the greenhouse shows that this is true. The writer has several times grafted staminate tops on carpellate stocks and vice versa without any effect upon the sex of the flowers, but does not mean to say that pollen which is produced by a carpellate type plant is the same genetically as that produced by a staminate plant. But since this paper is concerned only with the effect of environmental factors, a discussion of the hereditary aspects of the case will be deferred until a later paper.

PRELIMINARY OBSERVATIONS

The writer's attention was first directed to the effect of relative length of day and night upon sex in hemp by the peculiar behavior of several specimens which were being grown in the greenhouse during the late winter and early spring months. The seed was planted about the first of December and flower buds were first distinguishable on January 4. Relatively few flowers were produced and many of these were so abnormal that few seeds were matured. Following the maturity of this crop of seed the plants remained quiescent for several weeks and then resumed growth during the late spring months flowering again at about the normal blooming time for hemp which is grown in the open. It was thus possible to cause two crops of flowers to be produced in one year. The flowers which were produced during the second flowering period were normal and a fair crop of seed was matured. The same general effects are produced when hemp which has been planted in the open is moved into the greenhouse in the fall. The writer moved several such plants into the greenhouse before killing frosts occurred to ascertain if they could be held over in a semidormant condition. These plants dropped most of their leaves and made no additional growth for several weeks. During the month of December new leaves appeared and a small number of flowers were produced, most of which were abnormal. The production of flowers was still in progress when the plants were discarded late in January. This case refers only to carpellate forms, since the staminate plants die in late summer and apparently can not be kept alive by moving into the greenhouse.

The sexual abnormalities which appear during the short winter days are of the diverse kinds. The most common type among the plats has been the production of intersex flowers on plants of the staminate type. The individual flowers in

such cases vary all the way from pure staminate to pure carpellate. The writer has observed flowers containing two anthers and a rudimentary ovulary with one, two, or three stigmas; an apparently normal ovulary and two or three rudimentary anthers; three normal stamens and one terminated with a stigma; two ovularies, each with three stigmas; and many others. Of course, many of such abnormal flowers are not functional and therefore the highly intersex types seldom produce much pollen and set few seeds. Such behavior in the greenhouse during the winter when the daily period of light is relatively short indicates, as Schaffner (*8*) has already shown, that sex in hemp is largely under the control of environmental factors. If this is true it should be possible to obtain almost any desired degree of "sex reversal" by regulating the environmental factors.

PLAN OF THE EXPERIMENTS

For the purpose of producing different lengths of daily exposure to light, a series of ventilated chambers were used from which light could be excluded at will by means of sliding sashes upon a glass roof. The walls of each chamber were covered with black paper to prevent any light which might gain entrance from being reflected. Observation showed that when the shutters were closed these rooms were dark even at noon and gave a sufficiently good imitation of night for the purposes of this experiment. In the discussions which follow these chambers will be spoken of as the dark house. With such an arrangement, the large amount of labor connected with the daily moving of plants in and out of the house was eliminated and the effects of adverse weather conditions, such as high winds and rain, were reduced to a minimum.

The maximum difference in the temperature of the rooms did not exceed 3° C. during the course of the experiment. Although the difference between the temperature of the outside air and that inside the house was much greater than this, such differences are not of importance to this investigation, since adequate control plants were grown in one of the rooms. In work where the control plants were grown in the open it would, of course, be necessary to take into consideration the difference in temperature of the inside and the outside air.

The plants for this experiment were obtained from seed sown in flats in the greenhouse. As soon as the seedlings were well up, the flats were removed to the dark house and the different exposures to light were started at this time and continued until the maturity of the plants. At the time of potting, all of the seedlings were saved.

Inasmuch as soil and size of the pot have a marked effect on plant growth, great care was taken to keep these two factors constant for all lots of seedlings. Sufficient soil was thoroughly mixed at the start to last throughout the experiment. The potting of all lots was always done on the same day so that the plants in each house would have the same chance in this respect. The seedlings were first placed in 2-inch, later in 3-inch, and finally in 5-inch flower pots. To further prevent differences due to nutrition, all pots were placed on wooden benches so that the roots could not go through the bottom of the pots into soil.

Water was applied during the course of the experiment in such quantities as to keep the moisture content of the soil practically the same in all pots. Moisture tests were not made because it was believed that fluctuations in water supply within the limits present in this experiment have no effect upon sex in hemp.

The lengths of exposure used were 5 hours, 7 hours, 10 hours, and the normal length of day for this latitude. By reference to figure 1 it is seen that the period from sunrise to sunset during the course of the experiment was from 12 to slightly over 15 hours daily. The sashes were removed from all compartments at 7 a. m. and replaced as follows: In the 5-hour exposure at 12 m., in the 7-hour exposure

at 2 p. m., and in the 10-hour exposure as 5 p. m. Those which were given the full day exposure were treated in the same manner as the other plants except that they were not darkened.

In mentioning sex ratios the number of carpellate to 100 staminate plants has been used throughout this paper.

The effects produced by the various lengths of exposure to light are shown by the summary which is given in Table I.

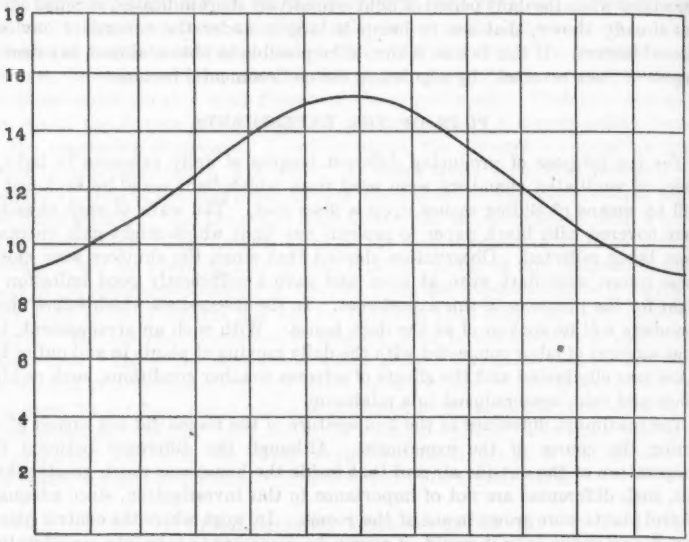

FIG. 1.—Graph showing the changes in length of day during the year in the latitude of Boston, Mass. Ordinates indicate 2-hour intervals of the day and abscissae indicate monthly periods of the year

TABLE I.—*Effect of day length on growth and reproduction*

Day length	Date planted	Budded	Sex ratio	Total number	Number died	Height
						Meters
5 hours	May 13	June 21	128:100	128	19	0.21
7 hours	...do....	June 14	119:100	101	20	0.50
10 hours	...do....	June 15	111:100	95	11	0.75
Day—inside	...do....	Aug. 13	115:100	73	23	2.05
Day—outside	...do....	July 14	140:100	120	4	1.04
Day—in plat	...do....	Aug. 14	97:100	133	----------	3.38
Total	----------	----------	117:100	650	82	----------

BEHAVIOR OF THE PLANTS

FIVE-HOUR EXPOSURE.—Plants which were exposed to light for only 5 hours daily showed very little development of either the vegetative or the reproductive parts. Side branches were absent and the leaves were few in number, small, and yellowish green in color. The number of flowers produced was very small and the maximum crop of seed matured by single carpellate plants was not over 12. Flower buds first appeared 38 days from the date of planting. The

sex ratio was 128:100 in a population of 128. At maturity the mean height of the entire lot was only 0.21 meter and the majority of the plants had to be supported. It is evident that this length of day approaches the lower limit in which hemp can be grown to the flowering stage.

SEVEN-HOUR EXPOSURE.—An increase of 2 hours daily over the 5-hour exposure not only caused the plants to develop buds 7 days sooner but also caused a significant increase in the development of vegetative and reproductive parts. The mean height attained was 0.50 meter and the stems were sufficiently strong to stand without support. The sex ratio was 119:100.

TEN-HOUR EXPOSURE.—The chief difference shown by this lot of plants was one of greater development. The mean height at maturity was 0.75 meter, leaves were large and deep green in color, and flowers were very abundant. Buds appeared 33 days from date of planting which is practically the same length of time required by plants in the 7-hour exposure to develop buds. The sex ratio was 111:100.

FULL DAY IN DARK-HOUSE.—The lot which was grown in the dark-house but exposed to the normal length of day for this latitude made so much growth that the size of the pot probably was a limiting factor in their development. The mean height at maturity was 2.05 meters. The main stem did not develop proportionally in thickness but remained slender and many of the plants were unable to support themselves at the time of flowering. The leaves were large and numerous but side branches were not produced to any extent. The time of flowering was greatly delayed in this lot by the long daily exposure to light, buds first appearing 84 days from the date of planting. The sex ratio was 115:100 in a population of 73.

FULL DAY OUTSIDE IN POTS.—A number of plants were grown in 5-inch pots in the open as a control on the lot which was grown in the dark-house. Flower buds appeared in 80 days from date of planting. Due to inability to properly control the moisture content of the soil, growth was somewhat irregular and the results obtained with this lot are not comparable with the results obtained with the other lots. The mean height at maturity was 1.04 meters and the sex ratio 140:100 in a population of 120.

PLANTED IN THE GARDEN PLAT.—Hemp which was grown in the garden plat attained a much larger size than did any of the potted plants. The mean height at maturity was 3.38 meters, although individual specimens reached a height of over 4.5 meters and a main stem diameter of over 1 inch. Side branches were produced in large numbers and flower production was very profuse. It is a significant fact that although this lot made a much greater vegetative development, they produced flower buds at the same time as did the lot which was grown in full-day exposure in the dark-house. This result indicates that the differences in development are due to factors other than light and that the time of flowering is not determined by the extent of vegetative development. The sex ratio of the plants in the garden plat was 97:100, the nearest to unity of any of the lots, but of course this is probably only a matter of coincidence.

EFFECT OF LENGTH OF EXPOSURE TO LIGHT ON GROWTH

The results show that both the rate and extent of growth, as well as the time of flowering, are greatly affected by the length of daily exposure to light. The effect on vegetative and sexual development can best be discussed separately.

TABLE II.—*Effect of length of exposure to light on growth*

Exposure	Date planted	Height	
		July 6	Aug. 15
		Meter	*Meters*
5 hours	May 13	0.22	0.47
7 hours	do	.46	.67
10 hours	do	.65	.90
Full day	do	.71	2.28

EFFECT ON VEGETATIVE DEVELOPMENT

Although exact measurements of the details of vegetative development in the dark-house were not taken, the mean heights give a certain measure of the effect of day length on the rate and the extent of growth. Plants in the 5-hour exposure group grew slowly at first which accounts for the small amount of growth made by the time of the first measurements on July 6. The data show that the extent of growth in all lots is roughly proportional to the duration of daily exposure to light. This does not mean, of course, that the rate of growth is proportional to the length of the daily exposure to light. The second series of measurements which were taken on August 15, show the same general effect of day length on height as the first measurements; the plants in the 5-hour day had the lowest mean height and those in the full daylight had the greatest mean height. Although it is realized that such results are not critical, they are very suggestive and agree with the work of Garner and Allard (*3*) who found that the increase in height of soy beans is proportional to the length of daily exposure to light.

Since the length of the growing period was greatly reduced by the accelerating action of the short exposure to light on the reproductive processes in the case of the plants which were grown in a short day, the amount of growth made by these lots was necessarily somewhat limited. The rôles played by the rate and the extent of growth, respectively, is a question which can not be answered from the data obtained in the dark-house. Another carefully controlled experiment has yielded results which show very clearly the relation between rate and extent of growth in hemp. Nine lots of 8 plants each were exposed to light daily for 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 7 hours, 12 hours, 18 hours, and 24 hours, respectively. Healthy seedlings of about the same height were selected and the treatment begun at the time of first potting. All seedlings were dark green in color and the mean height of each lot was 5 cm. The growth of each lot is represented graphically in figure 2. The rate of growth is not constant at all times but varies considerably at different periods. All lots began with a fairly low rate which gradually increased but this increase was not the same in all lots and it is due to this differential increase in growth rate that the extent of growth is not the same in all exposures at the end of a certain length of time. The lot which received 24 hours and that which received 18 hours of light, respectively, grew at so nearly the same rate that the data are represented by one curve. Their rate of growth was high from the start and it is due to the rapid growth during the first 10 days of the experiment that the height attained is greater than that of any of the other lots. Plants which were exposed to light for 12 and 7 hours daily were correspondingly slower in attaining a growth rate comparable to that of the 24-hour lot and their mean heights at the end of 34 days was correspondingly less.

Although the extent of growth was greatest in the longer light exposures, it does not necessarily follow that this result is due to a higher rate of growth over the entire period. In fact, the data show that in 7 or 12 hours of light daily the rate of growth at certain times equaled or even exceeded the rate of the 24-hour lot. The greater height at maturity in 18 or in 24 hours of light daily exposure

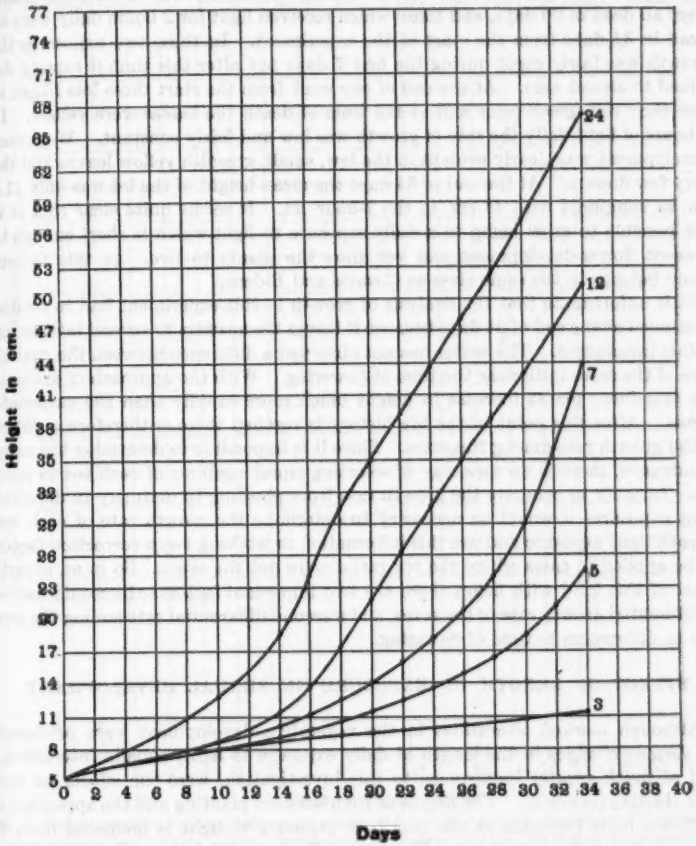

FIG. 2.—Graphs showing relation between relative length of day and night and growth in hemp. The seedlings were 5 cm. in height when the experiment was begun. Ordinates indicate growth in centimeters and the abscissas indicate the number of days. The numbers at the end of each graph show the number of hours of light received daily by each lot of plants. Measurements were made every two days

is due to the high rate during the early part of the growth period and the continuance of this rate over a long period of time. If the growth rate which the 7-hour plants have just prior to anthesis could be maintained over a long period they too would be tall but the period of this rapid rate of growth lasts but a few days and then rapidly declines to zero. Since hemp in this exposure grows relatively slowly up to the time of anthesis and then rapidly for only a short time, it necessarily follows that the plants will not attain a great height. The high growth

rate of hemp which is grown in a daily light exposure of 12 or more hours is continued up to the time of flowering, which is about 90 days from planting, and is then increased during anthesis. As a result such plants attain a relatively great height.

The minimum length of daily exposure to light required for continued growth of hemp in this experiment was 3 hours. In an exposure of 1 hour daily the plants were all dead in 20 days, and those which received light for 2 hours daily were all dead in 34 days from the start of the experiment. In these two exposures the growth was fairly rapid during the first 7 days but after this time it rapidly declined to almost zero. At the end of one week from the start these lots began to lose their rich green color and at the time of death the leaves were yellow. In 3 hours of light daily the rate of growth was low and fairly constant. Weakened development was clearly evident in the few, small, greenish yellow leaves and the very few flowers. At the end of 34 days the mean height of the lot was only 11.5 cm. as compared with 44 cm. in the 7-hour lot. It seems quite clear that it is not possible to grow hemp in a daily exposure to light which is short enough to prevent flower development and yet allow the plants to live. In this respect hemp belongs to the same class as Cosmos and Bidens.

It is unfortunate that the analysis of growth in this experiment had to be discontinued at the end of 34 days because it leaves the answers to several interesting points incomplete. The writer has not observed a difference between the growth rate of the sexes until near the time of flowering. With the approach of anthesis the staminate plants increase in height much more rapidly than the carpellate forms. After this point in the life history is reached there is therefore a differential growth rate among the sexes. Since it is impossible to determine the sexes in advance, there is no sure way of selecting equal numbers of each sex in each lot. In order to compare the growth rate from planting to maturity in different light exposures it would be necessary to determine the growth rate of each sex in each light exposure and use this information in working out a correction factor to be applied in cases where the sex ratios were not the same. So in an experiment of this kind with hemp there are two important factors to be considered—a differential growth rate of the sexes, and a second differential rate among the lots due to differences in time of flowering.

EFFECT OF LENGTH OF EXPOSURE ON SEXUAL DEVELOPMENT

Although marked alterations in the vegetative development were produced by various changes in the length of daily exposure to light, equally interesting, and of much greater importance to this investigation, were the effects on the reproductive processes. The length of time between planting and the appearance of flower buds increases as the length of exposure to light is increased from 7 hours daily, and an exposure of less than 7 hours daily also delays the time of blooming. The graphical representation of the data in figure 3 shows the relation between the time of flowering and the length of daily exposure to light. The criterion of time of flowering used here is that time when the sexual nature of the flower buds can be determined with certainty. The actual opening of the flowers does not occur until several days later. In 3 hours of light daily, which is the minimum for growth to maturity in hemp, flower buds appear in about 46 days from date of planting. As the length of daily exposure to light becomes greater, the time required for reaching the flowering stage becomes less until an exposure of 7 hours is reached. From this point on, the time between planting and flowering increases rapidly until it is about 90 days in a 15-hour day. Since the experiment in which hemp was exposed to continuous illumina-

tion was terminated long before the flowering stage was reached, it is not possible to say whether this lot would have bloomed sooner or later than those which were grown in the normal summer day length. The writer is inclined to believe that there would not be much difference. But with such a curve it is possible to predict the time that buds will appear with considerable accuracy, especially in the shorter exposures. Several times the writer has planted hemp when the

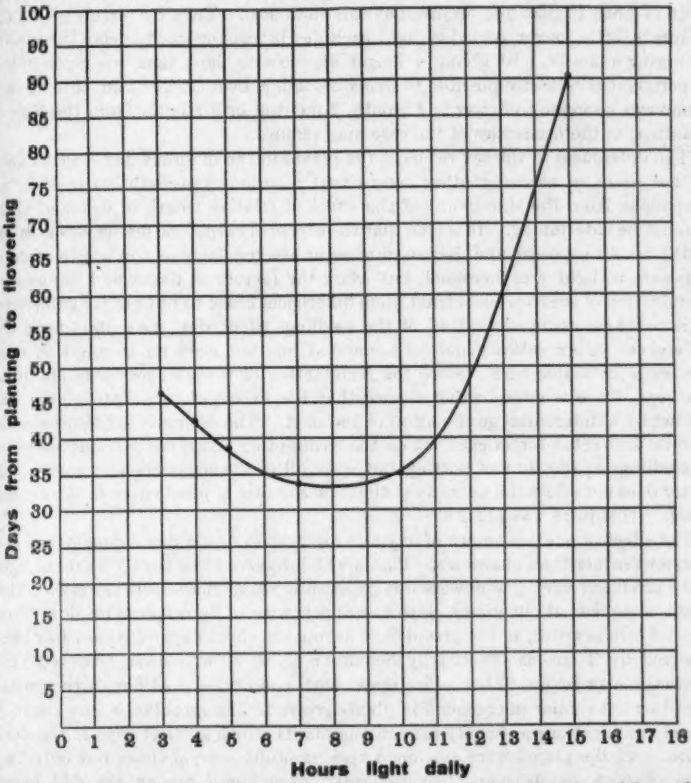

FIG. 3. Graph showing the relation between the number of days from planting to flowering and the number of hours of light received daily. Ordinates indicate 5-day intervals from the time of planting and abscissae indicate the number of hours of light received daily. Hemp can not be grown to the flowering stage in less than 3 hours of light daily

period of daily illumination was about 9 hours and has been able to predict within one day of the time when flower buds would appear.

An increase in the length of daily exposure to light after the flower buds appear has been found to greatly affect their further development. In an experiment carried on in the greenhouse during November and December it was found that flower production could be checked by increasing the period of illumination. Three plats were used. Plats two and three were given the normal daylight for that time of the year, but plat one was given, in addition, illumination from a 100-watt electric light from 4 to 8 p. m. every evening. On December 19, flower buds appeared on the plants in plats two and three. At this time the hemp in plat one

was still growing vigorously and no flower buds had appeared. Commencing on December 20, plat two was illuminated the same as plat one and in a few days the flower buds which had appeared began to show signs of being stunted. Many of these flowers never opened. All the plants in this plat were stimulated to further growth and no more flowers appeared until the period of illumination was shortened. The use of artificial illumination was discontinued on January 13, and on January 21 the hemp in plat two again showed flower buds. The appearance of buds in plat one occurred at this time also. Thus by merely giving the plants a little longer period of light each day it was possible to delay the time of flowering a month. By giving a longer exposure to light than was given in this experiment it is easily possible to delay blooming even more than this so that hemp can be made to flower in 1 month, 2 months, or 3 months from the time of planting, as the necessities of the case may require.

The differences in the sex ratios of the plants in the different day lengths may be the result of so many other causes that a serious consideration of the discrepancies from the standpoint of the effect of relative length of day and night can not be entertained. It is true that the excess of carpellate plants was greatest in the 5-hour exposure and decreased more or less regularly as the length of daily exposure to light was increased, but when the factors of death and differential germination of seed are considered, such differences cease to have any significance. If it could be assumed that all of the seedlings which died were staminate, the differences in sex ratios could be accounted for, but such an assumption does not seem justifiable here. Since the germination of the seed was only about 65 per cent, there is considerable chance that the excess of carpellate plants was caused by a differential germination of the seed. The sex-ratio differences could also be accounted for in each lot on the assumption of an unconscious selection of seedlings at the time of potting, but since all of the seedlings were saved, this factor does not affect the sex ratio of the total number of plants used in the experiment. This ratio was 117:100.

The effect of relative length of day and night upon flower development was one of extent rather than character. Plants which received less than 7 hours of light daily produced very few flowers but practically all of these were normal. This result is somewhat surprising since the appearance of hermaphrodite flowers on hemp which is grown in the greenhouse during the short days of winter has been reported by Tournois (*8*) and by Schaffner (*5*, *6*, *7*) who have observed that either sex may revert to the other under such conditions. Although the writer has observed similar phenomena in plants grown in the greenhouse, cases of such sexual modifications were very rare among plants grown in short days in the dark house. All the plants were examined very carefully several times but only two cases of such spontaneous "sex reversal" were found among the 650 hemp plants used in the experiment. One of these, and by far the more pronounced of the two, occurred on a staminate type which was growing in the normal length of day for that time of year. This plant produced a few hermaphrodite flowers on one of its branches. The other case was the appearence of a few abnormal staminate buds on a carpellate plant in the 10-hour exposure room. Only a few abnormal anthers were produced and these dried up before any pollen was liberated. These were the only cases of "sex reversal" among plants grown in the dark house and not otherwise treated. It seems quite clear, therefore, that a short exposure to light is not in itself the determining cause of sexual modification in hemp.

THE EFFECT OF MUTILATIONS ON SEXUAL DEVELOPMENT

The two factors which other investigators think are the most important in bringing about a modification of sexual expression in hemp are short daily exposure to light and flower removal. Schaffner (*5*, *6*, *7*) has been able to obtain various degrees of "sex reversal" by growing hemp in the greenhouse during the short days of winter and Pritchard (*4*) has shown that a certain amount of modification of the sexual state is apparently caused by removing the flower buds as soon as they appear. If such factors as length of day and flower removal influence the sexual condition of hemp, it seems reasonable to suppose that the two factors combined might have a decisive influence in causing "sex reversal." To test out this assumption, several plants were selected in each of the light exposures used in the dark house and their flowers were systematically removed during the flowering period.

From 16 staminate plants in the 5-hour exposure a total of 1,832 buds were removed but only two of these plants later developed any carpellate flowers. The hemp in this room was so weak that very few flowers were produced after the operation of flower removal, so that no very definite conclusions can be drawn from this experiment. The carpellate flowers that were produced were very abnormal and occurred in hermaphrodite buds.

From 14 staminate plants in the 7-hour day a total of 5,083 buds were removed. Subsequently, all but one of these plants developed carpellate flowers of various degrees of perfection. Many of these carpellate flowers were abnormal and such forms as a three or four-styled flower, anthers terminated by a style, and mixtures of stamens and carpellate flowers in the same bud, were very common. A certain number of functional carpellate flowers were produced and several seeds were matured.

From six staminate plants in the 10-hour exposure a total of 3,555 buds were removed but only three of these plants later developed any carpellate flowers. A few of these flowers were functional and matured a few seeds. From eight carpellate plants in this day length a total of 260 flowers was removed but only one of the plants so operated on later developed any staminate buds. Since, as already mentioned, one of the carpellate plants in this room developed some staminate buds without having had its flower buds removed, the above cited case of modification of the sexual expression by mutilition does not seem important.

The removal of flowers from hemp which was grown in the open during the long days of summer was without effect in causing modifications of the sexual state. From seven staminate plants a total of 16,425 buds was removed but none of these plants later developed any carpellate flowers. If the removal of the flowers was a determining factor in causing sexual modifications in this species, as Pritchard has suggested, some of these plants should have shown a tendency to develop carpellate flowers but their behavior in this experiment indicates that the mere removal of the flowers will not necessarily cause "sex reversal." In this connection the occasional spontaneous production of normal staminate flowers by carpellate plants in the field should be mentioned. Occasionally carpellate plants of any of the varieties of hemp will produce a few perfectly normal staminate flowers. This is especially true of the Simple Leaf variety. This fact makes the occasional production of a staminate bud by a mutilated carpellate plant entirely without significance and until more positive results are recorded we can conclude that the systematic removal of flowers from hemp which is grown in the field during the long days of summer does not affect the sex of the subsequent flowers.

To obtain more evidence on sexual modification in short days, additional experiments were conducted in the greenhouse during the winter with plants

grown from seed matured in the dark-house. The classification of the plants as to sex when they first bloomed showed 164 carpellate : 162 staminate. In a few days after the first flowers opened several hermaphrodite buds were observed, the phenomenon being more common among the staminate individuals. At once the plants were separated into several lots in order to study, in detail, the effect of flower removal during the short days on sexual development.

Early in the course of the experiments it became evident that the material used was sexually different from that used by Pritchard and Schaffner in their work. They state that the tendency is apparently from femaleness to maleness but in the material used in the writer's experiments the tendency appears to be from maleness to femaleness. Only 12.8 per cent of the carpellate plants developed any staminate buds while 53.12 per cent of the staminate plants developed some carpellate flowers. The extent of the change in sex varied greatly in different plants but there can be no doubt that the changes were by far more common among the staminate type.

Although flower removal from staminate hemp plants which are grown in a short daily exposure to light does apparently cause some of them to develop carpellate flowers, there seems to be always a certain number which do not show any sexual changes. For instance, in the above experiment the flower buds were removed from 32 staminate plants in one plat, with the result that 84.3 per cent of them later produced some carpellate flowers. Only 33.3 per cent of those in the control plat showed "sex reversal." In this particular case, then, about 16 per cent of the plants showed no tendency to develop flowers of the opposite sex. Since all of the plants were treated alike, it may be assumed that they are not all genetically identical as regards the sexual state.

A similar experiment on the effect of flower removal on the sexual development of carpellate plants showed less change among the test plants than among the controls. In the plat of 32 plants grown in the greenhouse during the winter, the flower buds were systematically removed as soon as they appeared with the result that 10.53 per cent of them later produced some staminate flowers. In the control plat 14.78 per cent produced some staminate flowers. It is very evident that flower removal from pistillate plants in this experiment has not resulted in significant sexual changes.

Pruning may indirectly have some effect on development but experimental results fail to show any significant modification of sex by this means. When the top of a staminate plant is removed at the time of anthesis, or shortly before, branches arise from the leaf axils and grow to a considerable length. Sometimes varying numbers of hermaphrodite flowers appear on such branches but the results of experiment show that this is probably only a matter of coincidence. In a plat of staminate plants from which the tops had been removed 50 per cent produced some hermaphrodite flowers. In the control plat 50 per cent also showed "sex reversal." In a plat of carpellate plants from which the tops had been removed 18.1 per cent developed hermaphrodite or staminate flowers, while 20 per cent in the control plat produced some staminate flowers. It seems quite clear, therefore, that such drastic pruning as cutting off the whole top of the plant does not significantly affect the sex of the plant.

DISCUSSION

After having observed thousands of hemp plants growing in the greenhouse and in the open, and after having tried many different kinds of stimulation in attempts to change the sex, the writer can not fully agree with the statement that the control of sex in hemp has reached a stage where it is possible to take a sample of seed and produce, at will, a stand of plants of any desired degree of sexual expression. It is true that Schaffner (*6*) has obtained as high as 88 per cent

of "sex reversal" in one plat which was grown in the greenhouse during the short days of winter, but when we consider that the plat contained only 16 plants, we must be careful in drawing the conclusion that the same percentage of plants in a much larger plat would show the same changes. Until it is possible to obtain the same degree of sex modification in several plats grown under the same conditions, only tentative conclusions can be drawn concerning the exact control of sexual expression in hemp. It may well be that the genetic balance in different hemp plants is such that a unit change in the environmental factors will produce more visible change in the sex of some than of others. That this balance can be upset and various sexual changes produced by varying the length of daily exposure to light there can be no doubt; but to say that any desired degree of sexual change can be obtained by this means is quite another thing.

The general term "sexual reversal" which has been used by investigators to designate a modification of sexual expression is not entirely a satisfactory one. Pritchard (*4*) and Schaffner (*6*, *7*) have used the term to include all those plants which were first either pure carpellate or pure staminate, but which later produced some flowers of the opposite sex. In the majority of cases these secondary flowers are intersexual and in many cases entirely sterile. Such cases, in the strict sense of the word, are only modifications of the sex of a few flowers. A plant can not be said to have completely reversed its sex until it produces exclusively flowers of the opposite sex. It will be recognized that such a condition is rather rare in any of the so-called dioecious species. Schaffner (*5*, *6*, *7*) has undoubtedly obtained many instances of modification of the sexual expression but not many cases of "sex reversal" in the sense that the plants which were one sex at first produced nothing but flowers of the opposite sex later in their life. A few cases which approach this degree of sex change have been obtained by the writer, however. At least two of the plants which were at first apparently pure staminate, later in their lives produced nothing but carpellate flowers and matured seed. Such cases are as near a complete reversal of sex as probably can be obtained in hemp.

At the present time so little is known concerning the genetic complex of dioecious species of plants that it is not easy to draw conclusions concerning the behavior of the various intersex types. It is known that sex in hemp is somehow influenced by the environment, especially by the relative length of day and night, and also known that the time of flowering of this species is almost entirely controlled, within certain limits, by this same factor. But since the dimorphic vegetative types occur in about equal numbers regardless of the environment there must be something genetic concerned and the fact that one or both of these types later in life produce flowers of the opposite sex, is not necessarily a valid argument that genetic factors are not concerned. Many characters which are genetic in nature are affected by various environmental factors and there is no reason why sex can not be affected likewise. But since the genetics of this particular case is to be published in a later paper, further discussion of the point will not be entered into here.

SUMMARY

1. Hemp possesses a distinct vegetative and sexual dimorphism, but either sex may, under certain conditions, produce flowers of the opposite sex. The vegetative differences are the same for the plants which remain sexually pure and those which do not.

2. The ultimate height attained by hemp appears to be due largely to the length of the period of rapid rate of growth. This period of rapid rate of growth seems to be roughly proportional to the length of daily exposure to light. In a long daily light exposure the high growth rate extends over a long period and the plants are tall at maturity, but in a short daily exposure to light the period

of high growth rate is correspondingly shorter and the plants attain a height of only 1 to 2 feet. The growth rate of hemp grown in a short daily exposure to light may, however, at some time in its life exceed the growth rate of hemp grown in longer daily exposures.

3. The time of flowering of hemp is largely controlled by the relative length of day and night. A daily exposure to light of 7 hours seems to produce the greatest acceleration of the flowering process. A longer or a shorter daily exposure produces a retarding effect on flower development.

4. Three hours of light daily is the least in which hemp will grow for any length of time. If a shorter period of daily illumination is used the plants die before reaching the flowering stage.

5. It is not possible to retard the length of daily period of illumination sufficiently to prevent flowering without causing the death of the plants.

6. "Sex reversal" does not necessarily result when hemp is grown in the greenhouse during the winter months. More intersex types are found under such conditions, but many of the plants remain sexually pure. We may conclude that environment in some way affects the development of sex in this species, but the evidence shows that it does not control it.

7. Removal of flowers does not necessarily result in sexual modifications in hemp, although a few plants so treated and grown in a short daily exposure to light reversed their sex to the extent that some apparently pure staminate individuals matured seed. The occurrence of this phenomenon was not sufficiently general, however, to allow definite conclusions to be drawn. The removal of flowers from hemp which is grown in the field is without effect in causing sexual changes.

8. Although the development and expression of sex in hemp are affected by environmental factors, the changes produced are in many cases relatively minor ones, and a sweeping conclusion that genetic factors are in no way concerned with sex in this species is not warranted at the present time.

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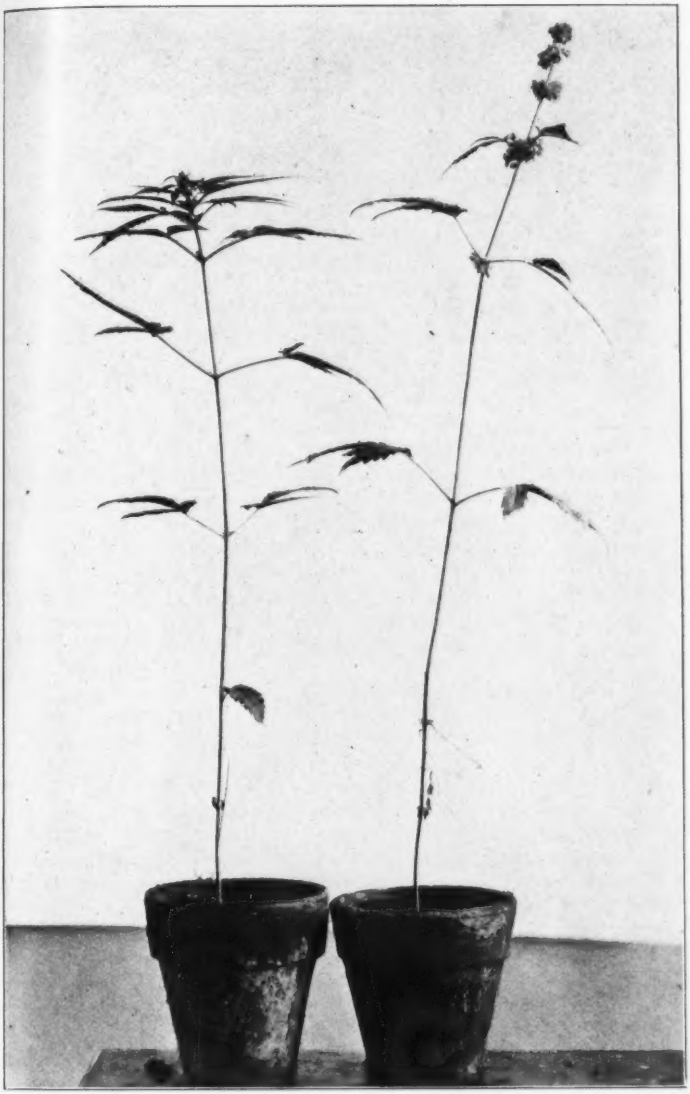
PLATE 1

Right: Staminate hemp plant grown in the greenhouse during the short days of winter. Note clustering of flowers and scarcity of leaves at the top.

Left: Carpellate hemp plant from seed planted at the same time as the plant on the right. The clustering of leaves at the tip and the inconspicuousness of the flowers are characteristic of the carpellate type.



SPACING EXPERIMENTS WITH ACALA COTTON IN SOUTHERN CALIFORNIA[1]

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Different spacings of Upland cotton under irrigation in the Coachella Valley of California, which is located in Riverside County northwest of the Salton Sea, were compared in 1922 and 1923. Most of the cotton lands of the Coachella Valley are below sea level and the summer months are very hot. Though the extremely high temperatures prevailing during the summer months often cause excessive shedding of squares and young bolls, the season is so long that the plants are enabled to continue fruiting over a long period and large yields are usually obtained. Fields of Acala cotton planted as late as the tenth of June have sometimes yielded two bales to the acre.

In the Eastern cotton belt, where the crop season may be very short on account of dry weather, early frost, or boll-weevil damage, the ability of closer spaced plants to set a good crop in a short period is an important advantage. But in the irrigated valleys, where high temperatures interfere with the setting of an early crop, a long-continued fruiting period is necessary to obtain good yields. Under such conditions, it might be supposed that wide spacing, which permits the plants to grow large, would be an advantage. However, direct comparisons of spacings with the Acala variety of Upland cotton grown in the Coachella Valley indicate no advantage from wider spacing, even under the long-season conditions.

The usual practice in the valley is to chop the cotton out from 12 to 16 inches in the row. Spacing experiments were conducted in 1922 and 1923 to compare plants spaced at 12 and 6 inches in the row with "unthinned" cotton. As a rather heavy rate of seeding was used, thick stands were obtained and some plants had to be removed from the unthinned rows in order to prevent crowding where the stand was "bunchy." The plants were left 2 to 4 inches apart in the row, and this spacing has been designated as "2-inch" in the accompanying figures and tables. In thinning the 6 and 12 inch rows a measure was used and the plants were left as near these distances apart as the stand would permit.

The designated spacings indicate the distance between the majority of the plants rather than the average distance between plants, which is somewhat greater on account of occasional short skips in the stand. The plant counts contained in Table III, however, show the spacings to be very close to that designated, except in the case of the 2-inch spacing. The 6-inch spaced blocks contain about twice as many plants as the 12-inch blocks, and the 2-inch blocks contain about twice as many plants as the 6-inch blocks, so that the average spacing of blocks referred to as "2-inch" was about 3 inches, though much of the cotton was as close as 2 inches.

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SPACING EXPERIMENT IN 1922

The experimental material was planted May 17, 1922. The soil used was a fairly fertile sandy loam and quite uniform. The plants were thinned June 14 and 15, when they were 6 to 8 inches tall and had five to seven true leaves. To afford the best conditions for the 2-inch spacing it might have been better to reduce the stand in these rows when the plants were smaller, since that degree of crowding is sufficient to suppress the vegetative branches.

The experiment consisted of 24 rows 4 feet apart and 375 feet long. The 24 rows were grouped into five 4-row blocks with 2 guard rows on each side of the test to protect the outer blocks from "outside row effect." In blocks No. 1, 3, and 5 the plants were approximately 2 inches apart, while in block No. 2 the plants were spaced 6 inches apart and in block No. 4, 12 inches apart.

The plants were irrigated as would be good practice for a commercial field, without reference to the different spacings, as no difference in the water requirements of the different spacings was noticed. The plants grew to be from 4 to 5½ feet tall, the taller plants being on the east and west sides of the field.

The first picking was made October 10 and 11, which was somewhat too late to show differences in the earliness of the different spacings. The second picking was made December 20, about two weeks after the first frost.

In picking, the test was divided across the rows into three sections, thus making it possible to obtain six direct comparisons of the 6- and 12-inch spacing with the 2-inch spacing by comparing each section of the 6- and 12-inch spaced blocks with the section of 2-inch spacing on either side. These three sections are designated as A, B, and C, the length of rows in each section being 100, 125, and 150 feet respectively. The rows were not divided into sections of equal length because the plants on the east and west sides of the field were somewhat larger than those in the middle section, and the divisions were made so as to incorporate the larger plants in sections A and C and the smaller plants in section B.

The weight of the first and second pickings and the total yields from each section of each row are presented in Table I. The yields also are shown graphically in figure 1.

Six side-by-side comparisons of the 6 with the 2-inch spacing are possible by comparing the 6-inch plants in each of the three sections of block 2 with the 2-inch plants in the three sections of blocks 1 and 3, on either side.

In the first picking, made October 10, in five out of the six comparisons the 2-inch spacing outyielded the 6-inch, while in the second picking the 2-inch spacing led in only three of the six comparisons. In total yield, however, the 2-inch outyielded the 6-inch in five of the six comparisons.

Comparison of the total yields from each section of blocks No. 1, 2, and 3 shows that there was little difference in yield of the 6-inch and 2-inch spacings. The three sections of block No. 2 where plants were spaced 6 inches apart, yielded 63.0, 74.8, and 107.5 pounds, respectively, giving a total of 245.3 pounds for the entire block. The yields from each section of the two adjoining blocks of 2-inch spaced plants were as follows: Block No. 1, 64.2, 79.5, and 112.7 pounds, with a total of 256.4 pounds; block No. 3, 62.5, 75.6, and 110.8 pounds, with a total of 248.9 pounds. Thus in block No. 1 each section of 2-inch spaced plants outyielded the adjacent sections of 6-inch spacing, giving a total gain of 11.1 pounds for the entire block of 2-inch spaced plants. The 2-inch spacing in block No. 3 also outyielded the 6-inch spacing, but the gain was only 2.6 pounds.

Six comparisons are possible also between the 12- and 2-inch spacing, by comparing the 12-inch spaced plants in the three sections of block No. 4 with the

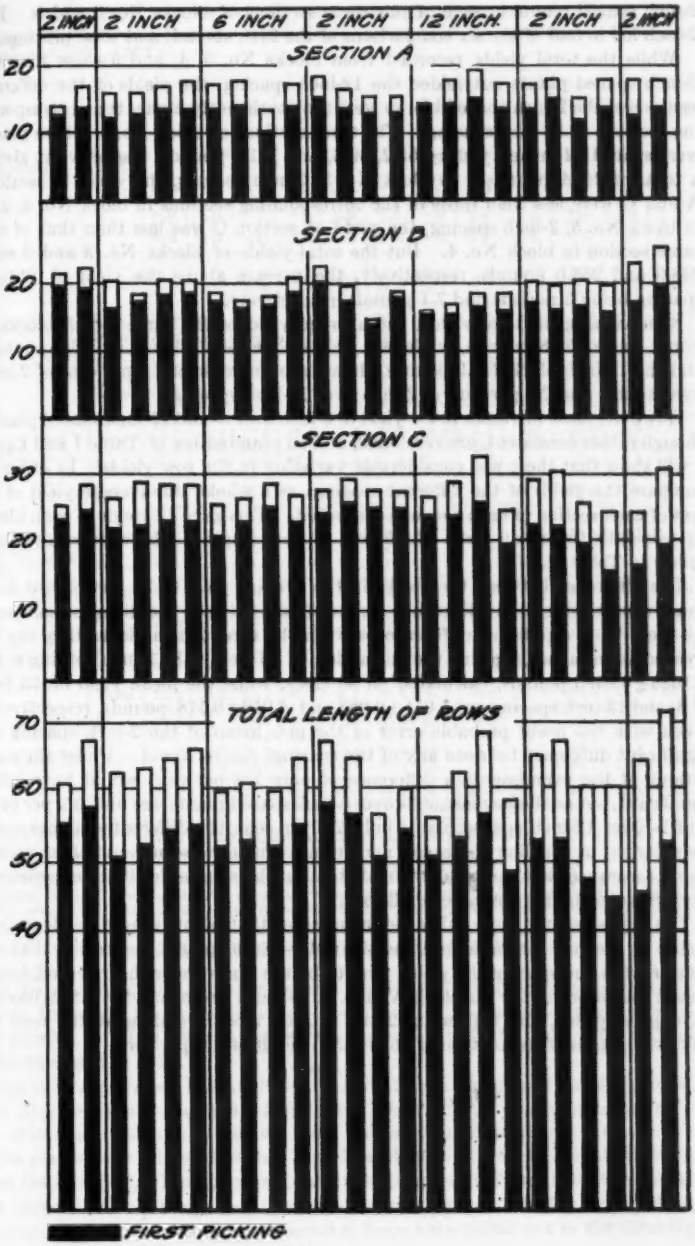

FIG. 1.—Yield in pounds of the first and second picking by sections and total length of rows, spacing experiment, Coachella Valley, Calif., 1922

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2-inch spaced plants in each of the three sections of blocks No. 3 and 5. The 2-inch led in half of the six comparisons of the first, second, and total pickings.

While the total yields recorded from blocks No. 3, 4, and 5 show that the 2-inch spaced plants outyielded the 12-inch spacing, the yields of the different sections of the 2-inch spaced blocks were not consistently higher than corresponding sections of 12-inch spacing. The three sections of block No. 4, where plants were spaced 12 inches, yielded 65.2, 69.5, and 111.2 pounds, respectively, giving a total of 245.9 pounds. In block No. 3, 2-inch spacing, the yields of sections A and C were less than those of the corresponding sections in block No. 4, and in block No. 5, 2-inch spacing, the yield of section C was less than that of the same section in block No. 4. But the total yields of blocks No. 3 and 5 were 248.9 and 253.0 pounds, respectively, the increase above the yield of 12-inch spacing being 3 pounds and 7.1 pounds, respectively.

Summarizing the total yields, the average yield of the three 2-inch blocks is found to be 252.8 pounds, while the yield of the 6-inch block is 246.3 pounds, and the 12-inch block 245.9 pounds, showing an increase of 3.2 per cent of 2-inch over 6-inch, and 2.8 per cent of 2-inch over 12-inch spacing.

However, these increases in the yield of 2-inch over 6- and 12-inch spaced plants, though rather consistent, are very small and an examination of Table I and figure 1 will show that there was considerable variation in the row yields. In order to compare the yields of the different spacings as a whole, the average yield of 25 feet of each section of each row was computed. This gave 12 yields in each block to calculate the mean yield of 25 feet of row length. These determinations follow in Table II.

The difference between the blocks in the average yield of 25 feet of row is so small that no significant difference can be shown between the different spacings. As there is no significant difference between the three 2-inch blocks they can be treated as one array, showing the mean yield of 25 feet of the 2-inch spacing to be 4.1683 ± 0.0485 pounds, calculated on 36 cases, while the mean yield of 25 feet of 6- and 12-inch spacing are 4.051 ± 0.083 and 4.059 ± 0.118 pounds, respectively. Even with the lower probable error of the new mean of the 2-inch spacing no significant difference between any of the spacings can be found. Under the conditions of the experiments a difference of only 7.5 per cent would have been significant, but as the increase of 2- over 6-inch spaced plants was only 3.2 per cent and 2- over 12-inch spaced plants only 2.8 per cent, the different spacings must be regarded as yielding the same, and from a biometric standpoint there would be no assurance that the results would stand in the same order if the experiment were repeated under the same conditions.

The yields secured from this experiment show that plants spaced as close as 2 inches in the row yielded as well as the wider-spaced plants, indicating that no matter what the spacing the yields tend to be the same under the irrigated, long-season conditions of the Coachella Valley. It should also be noted that all blocks gave good yields, the highest, a 2-inch spaced block, yielding at the rate of 1,958.56 pounds of seed cotton or about 653 pounds of lint per acre.

TABLE I.—*Yields of the first, second, and total pickings by sections of rows, total length of rows, and four-row block totals, cotton spacing experiment, Coachella Valley, Calif., 1922* [a]

Spacing	Row No.	Section A 100 feet			Section B 125 feet			Section C 150 feet			Total length of row		
		Yields			Yields			Yields			Yields		
		First picking	Second picking	Total pounds	First picking	Second picking	Total pounds	First picking	Second picking	Total pounds	First picking	Second picking	Total pounds
2-inch	Guard	12.5	1.3	13.8	19.7	2.1	21.8	23.5	2.2	25.7	55.7	5.7	61.5
		13.7	2.8	16.6	19.2	3.3	22.6	24.7	3.6	28.3	57.7	9.8	67.6
Block No. 1, 2-inch.	1	12.0	3.5	15.5	16.7	3.7	20.5	22.0	4.5	26.5	50.7	11.7	62.5
	2	12.7	3.0	15.7	17.0	1.6	18.6	22.5	6.5	29.0	52.2	11.1	63.3
	3	13.5	2.5	16.0	18.0	2.1	20.1	23.2	5.3	28.6	54.7	10.0	64.7
	4	13.5	3.5	17.0	18.5	1.8	20.3	23.0	5.6	28.6	55.0	11.0	66.0
	Total	51.7	12.5	64.2	70.2	9.2	79.5	90.7	21.9	112.7	212.6	43.8	256.5
Block No. 2, 6-inch.	1	13.2	3.0	16.2	17.5	.8	18.3	21.2	4.6	25.8	52.0	8.5	60.5
	2	13.7	3.6	17.3	16.2	1.2	17.5	23.0	3.1	26.1	53.0	8.0	61.0
	3	10.0	3.2	13.2	16.7	1.2	18.0	25.2	3.6	28.8	52.0	8.1	60.1
	4	13.7	2.6	16.3	18.7	2.2	21.0	22.5	4.3	26.8	55.0	9.2	64.2
	Total	50.6	12.4	63.0	69.1	5.4	74.8	91.9	15.6	107.5	212.0	33.8	245.8
Block No. 3, 2-inch.	1	14.5	3.0	17.5	20.2	2.3	22.6	24.0	3.3	27.3	58.7	8.7	67.5
	2	13.2	2.6	15.8	17.5	2.2	19.7	25.7	3.2	29.0	56.5	8.1	64.6
	3	12.5	2.2	14.7	14.0	.6	14.6	24.2	3.2	27.5	50.7	6.1	56.8
	4	12.5	2.0	14.5	18.0	.7	18.7	24.2	2.7	27.0	54.7	5.5	60.2
	Total	52.7	9.8	62.5	69.7	5.8	75.6	98.1	12.4	110.8	220.6	28.4	249.1
Block No. 4, 12-inch.	1	11.5	2.2	13.7	15.5	.5	16.0	23.5	2.7	26.2	50.5	5.5	56.0
	2	13.7	3.1	16.8	15.7	1.3	17.1	23.7	5.0	28.7	53.2	9.5	62.7
	3	14.5	3.7	18.2	16.5	1.8	18.3	25.5	6.6	32.1	56.5	12.2	68.7
	4	13.2	3.3	16.5	16.0	2.1	18.1	19.5	4.7	24.2	48.7	10.3	59.1
	Total	52.9	12.3	65.2	63.7	5.7	69.5	92.2	19.0	111.2	208.9	37.5	246.5
Block No. 5, 2-inch.	1	13.0	3.1	16.1	17.2	2.7	20.0	22.5	6.5	29.0	52.7	12.3	65.1
	2	14.5	2.7	17.2	16.0	3.8	19.8	22.0	8.0	30.0	52.5	14.6	67.1
	3	11.7	2.7	14.4	16.5	3.8	20.3	19.5	6.2	25.7	47.7	12.8	60.6
	4	13.2	3.5	16.7	15.7	4.0	19.7	15.7	8.3	24.1	44.7	15.8	60.6
	Total	52.4	12.0	64.4	65.4	14.3	79.8	79.7	29.0	108.8	197.6	55.5	253.1
2-inch	Guard	12.0	4.7	16.7	17.2	5.8	23.1	16.2	8.0	24.2	45.5	18.6	64.1
		13.2	3.0	16.2	19.5	5.5	25.0	19.5	10.8	30.3	52.2	19.3	71.6

[a] The weights were recorded to hundredths of pounds, but the yields are given in tenths of pounds. In computing the total yield hundredths of pounds were included if they equaled or exceeded a tenth of a pound; hence the total row yields in some instances are slightly greater than the sum of the three sections.

SPACING EXPERIMENT IN 1923

In the season of 1923, another spacing experiment was conducted with Acala cotton, the same plant spacings being compared. The experimental material was planted on April 23, and located on approximately the same plot of land which was used in 1922.

The 1923 experiment had additional 6 and 12-inch blocks and consisted of 32 rows 3½ feet apart and 370.5 feet long. Acala cotton was planted on each side of the test, which protected the outer blocks from "outside row effects."

The plants were thinned between May 25 and June 5, the 2-inch spaced blocks being thinned first, when the plants were 8 to 10 inches tall and had about eight true leaves. Tomosis was very prevalent at the time of thinning and quite a number of plants having aborted terminal buds were pulled out in the thinning process where possible.

In the first four rows the plants were spaced 12 inches apart, the next four 2-inch, the next four 6-inch, and the next four 2-inch spacings were used, repeated once, making eight 4-row blocks in all. Thus blocks No. 1 and 5 were thinned to 12 inches apart, blocks No. 3 and 7 to 6 inches, and blocks No. 2, 4, 6, and 8 to 2 inches.

As in the 1922 test, this experiment was irrigated without reference to the different spacings. On one or two occasions, however, the whole test suffered somewhat for lack of water as the supply was not quite adequate during the hot summer months. The majority of the plants grew to be from 4½ to 5½ feet in height.

TABLE II.—*Mean yield of 25 feet of row length in each block*

Block No.	Spacing	Pounds
1	2-inch	4.225±0.071
2	6-inch	4.051± .083
3	2-inch	4.099± .101
4	12-inch	4.059± .113
5	2-inch	4.181± .067

Yields of the first, second, and total pickings are given by sections of rows, total length of rows, and 4-row block totals in Table III. Row yields of the first and second picking by sections and total length of rows are shown graphically in figure 2. The first picking was made September 21 to 24, which was early enough to show to some extent the relative earliness of the different spacings. The second picking was made December 20 and 21.

Nine direct comparisons are possible between the 2- and 12-inch spacings by comparing the yields of 12-inch spaced plants in each of the three sections of blocks No. 1 and 5 with the yields of 2-inch spaced plants in each of the 3 sections of blocks No. 2, 4, and 6 which are adjacent to the 12-inch blocks.

In the first picking the 2-inch spaced plants led in only five of the nine comparisons, but in the second picking the 2-inch spaced plants led in eight of the nine comparisons. In total yield the 2-inch also led in eight of the nine comparisons.

Twelve direct comparisons are possible between the 2- and 6-inch spacings by comparing the yields of 6-inch spaced plants in each of the three sections of blocks No. 3 and 7 with the yields of 2-inch spaced plants in each of the three sections of blocks No. 2, 4, 6, and 8, which are adjacent to the 6-inch blocks.

In the first picking the 2-inch spaced plants led in only five of the twelve comparisons and in one comparison the yields of adjacent 2- and 6-inch blocks were identical. In the second picking the 2-inch spaced plants led in ten, and in the total picking in nine of the twelve comparisons.

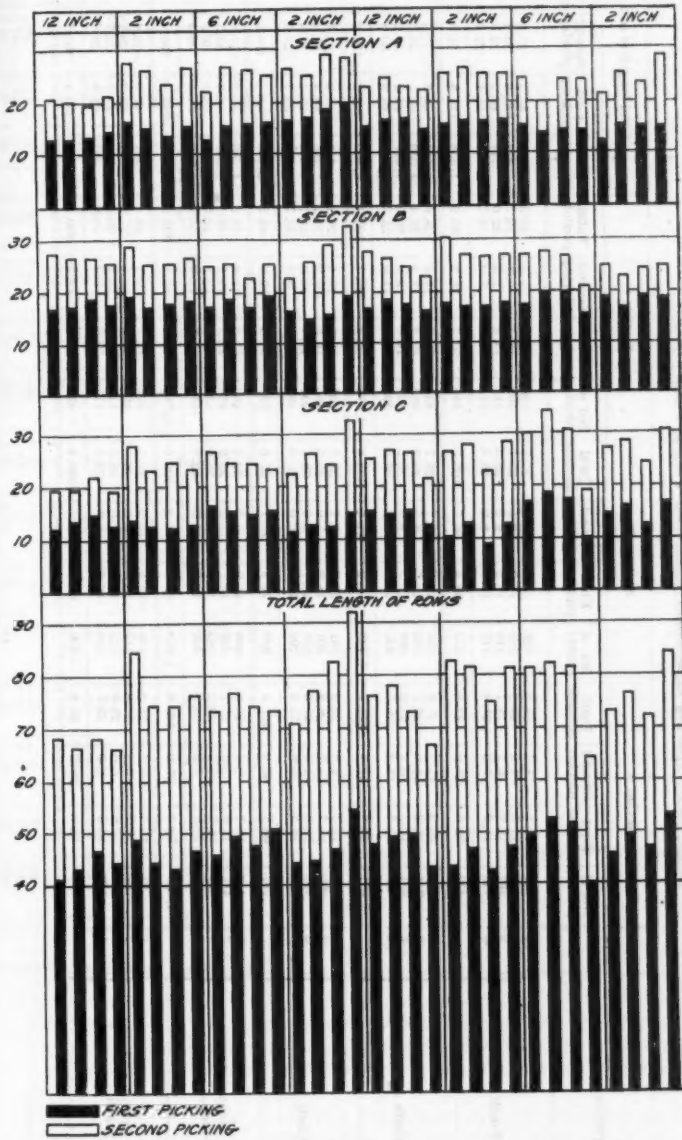

FIG. 2.—Yield in pounds of the first and second picking by sections and total length of cotton rows, spacing experiment, Coachella Valley, Calif., 1923

TABLE III.—*Number of plants and yields of the first, second, and total picking by sections of rows, total length of rows, and four-row block totals, cotton spacing experiment, Coachella Valley, Calif., 1923*

Spacing	Row No.	Section A				Section B				Section C				Total length of row			
		No. of plants	First picking	Second picking	Total pounds	No. of plants	First picking	Second picking	Total pounds	No. of plants	First picking	Second picking	Total pounds	No. of plants	First picking	Second picking	Total pounds
Block No. 1, 12-inch	1	111	12.7	8.5	21.2	120	16.5	11.2	27.7	108	12.0	7.5	19.5	339	41.2	27.2	68.4
	2	111	12.5	7.7	20.2	119	17.0	9.5	26.5	109	13.5	6.2	19.7	339	43.0	23.4	66.4
	3	107	13.2	6.5	19.7	119	18.5	8.0	26.5	118	14.5	7.5	22.0	344	46.2	22.0	68.2
	4	110	14.2	7.2	21.4	115	17.5	8.0	25.5	111	12.7	7.0	19.7	336	44.4	22.2	66.6
Total		439	52.6	29.9	82.5	473	69.5	36.7	106.2	446	52.7	28.2	80.9	1,358	174.8	94.8	269.6
Block No. 2, 2-inch	1	391	16.2	11.7	27.9	454	19.0	9.7	28.7	435	13.5	14.5	28.0	1,280	48.7	35.9	84.6
	2	354	15.2	11.0	26.2	363	16.7	8.5	25.2	333	12.2	11.2	23.4	1,050	44.1	30.7	74.8
	3	431	13.7	10.2	23.9	488	17.5	8.0	25.5	408	12.0	13.0	25.0	1,327	43.2	31.2	74.4
	4	455	15.5	11.2	26.7	443	18.0	8.2	26.2	395	12.7	11.0	23.7	1,293	46.2	30.4	76.6
Total		1,631	60.6	44.1	104.7	1,748	71.2	34.4	105.6	1,571	50.4	49.7	100.1	4,950	182.2	128.2	310.4
Block No. 3, 6-inch	1	195	12.7	9.5	22.2	231	17.0	7.7	24.7	207	16.2	10.5	26.7	633	45.9	27.7	73.6
	2	200	15.7	11.2	26.9	201	18.2	7.0	25.2	185	15.5	9.2	24.7	586	49.4	27.4	76.8
	3	196	16.0	11.2	27.2	216	16.7	6.0	22.7	202	14.7	10.0	24.7	614	47.4	27.2	74.6
	4	206	16.2	9.0	25.2	201	19.0	6.0	25.0	193	15.5	8.2	23.7	600	50.7	23.2	73.9
Total		797	60.6	40.9	101.5	849	70.9	26.7	97.6	787	61.9	37.9	99.8	2,433	193.4	105.5	298.9
Block No. 4, 2-inch	1	412	16.7	9.7	26.4	448	16.0	6.5	22.5	400	11.5	11.0	22.5	1,260	44.2	27.2	71.4
	2	417	17.0	8.2	25.2	442	14.7	11.2	25.9	438	12.7	13.7	26.4	1,297	44.4	33.1	77.5
	3	391	18.7	10.5	29.2	460	15.5	13.5	29.0	435	12.2	12.5	24.7	1,286	46.4	36.5	82.9
	4	316	20.0	8.2	28.2	463	19.2	13.0	32.2	440	15.0	17.2	32.2	1,219	54.4	38.4	92.8
Total		1,536	72.4	36.6	109.0	1,813	65.4	44.2	109.6	1,713	51.4	54.4	105.8	5,062	189.4	135.2	324.6
Block No. 5, 12-inch	1	91	15.7	7.5	23.2	116	16.5	11.2	27.7	104	15.7	9.7	25.4	311	47.9	28.4	76.3
	2	107	16.7	8.2	24.9	117	18.0	8.0	26.0	107	14.5	12.7	27.2	331	49.2	28.9	78.1
	3	103	17.0	6.0	23.0	112	17.2	7.0	24.2	115	15.7	10.7	26.4	330	49.9	23.7	73.6
	4	106	14.7	8.0	22.7	114	16.0	6.5	22.5	113	12.7	9.2	21.9	333	43.4	23.7	67.1
Total		407	64.1	29.7	93.8	459	67.7	32.7	100.4	439	58.6	42.3	100.9	1,305	190.4	104.7	295.1

Block No. 6, 2-inch	1	389	15.7	10.0	25.7	458	17.5	12.5	30.0	500	19.2	16.7	26.9	1,347	43.4	39.2	82.6
	2	398	16.7	10.0	26.7	441	16.7	10.0	26.7	410	13.0	15.0	28.0	1,249	46.4	35.0	81.4
	3	386	16.2	9.2	25.4	443	17.0	9.5	26.5	411	9.0	14.2	23.2	1,240	42.2	32.9	75.1
	4	383	16.7	9.2	25.9	411	17.7	9.2	26.9	399	12.7	16.0	28.7	1,193	47.1	34.4	81.5
Total		1,556	65.3	38.4	103.7	1,753	68.9	41.2	110.1	1,720	44.9	61.9	106.8	5,029	179.1	141.5	320.6
Block No. 7, 6-inch	1	194	15.7	9.0	24.7	209	17.2	9.7	26.9	200	17.0	13.0	30.0	608	49.9	31.7	81.6
	2	216	14.0	6.2	20.2	216	19.5	8.0	27.5	200	18.7	15.7	34.4	632	52.2	29.9	82.1
	3	214	14.7	9.7	24.4	215	19.5	6.7	26.2	203	17.7	13.2	30.9	632	51.9	29.6	81.5
	4	222	14.5	9.7	24.2	211	15.5	5.0	20.5	199	10.0	9.7	19.7	632	40.0	24.4	64.4
Total		846	58.9	34.6	93.5	851	71.7	29.4	101.1	802	63.4	51.6	115.0	2,499	194.0	115.6	309.6
Block No. 8, 2-inch	1	378	12.0	9.5	21.5	447	18.7	6.0	24.7	428	15.0	12.5	27.5	1,253	45.7	28.0	73.7
	2	448	15.5	10.0	25.5	372	17.7	5.0	22.7	382	16.2	12.5	28.7	1,202	49.4	27.5	76.9
	3	389	15.2	8.5	23.7	441	19.0	5.0	24.0	401	12.7	12.0	24.7	1,231	46.9	25.5	72.4
	4	378	18.2	10.7	28.9	342	18.5	6.0	24.5	403	17.2	13.5	30.7	1,123	53.9	30.2	84.1
Total		1,593	60.9	38.7	99.6	1,602	73.9	22.0	95.9	1,614	61.1	50.5	111.6	4,809	195.9	111.2	307.1

The comparisons show that in the first picking the different spacings gave about the same yield, and that 2-inch spaced plants outstripped the wider spaced plants in the second picking, which gave the 2-inch spaced plants a greater total yield.

Comparisons of the total block yields show that the two blocks of 12-inch spaced plants were outyielded by three adjacent blocks of 2-inch spaced plants 15.1, 9.9, and 8.6 per cent, respectively. The increase of 2-inch over 6-inch spaced blocks was not so great. The two blocks of 6-inch spaced plants were outyielded by three adjacent blocks of 2-inch spaced plants 3.8, 8.5, and 3.5 per cent, respectively, but in one instance (block No. 8) an adjacent block of 2-inch spacing gave a decrease of 0.8 per cent. However, the mean yield of the four 2-inch spaced blocks is 315.67 pounds, of the two 6-inch blocks 304.25 pounds, and of the two 12-inch blocks 282.35 pounds, showing an increase of 3.7 per cent of 2-inch over 6-inch spaced plants, 11.8 per cent of 2-inch over 12-inch spaced plants, and an increase of 6-inch over 12-inch spaced plants of 7.8 per cent.

All spacings, however, gave good yields, the average production of the 2-inch spacing being at the rate of 2,651 pounds of seed cotton, or about 883 pounds of lint, which is a little over a bale and three-quarters per acre.

There are four rows 123.5 feet long in each of the three sections of each block, and mean yields of the first, second, and total picking of the twelve 123.5-foot rows in each block are given in Table IV.

TABLE IV.—*Mean yield of twelve 123.5-foot row sections of blocks of 2-, 6-, and 12-inch spaced plants, Coachella Valley, Calif., 1923*

Block No.	Spacing	First picking	Second picking	Total yield
		Pounds	*Pounds*	*Pounds*
1	12-inch	14.56±0.42	7.90±0.16	22.48±0.59
2	2-inch	15.18± .44	10.68± .36	25.87± .33
3	6-inch	16.12± .30	8.79± .34	24.91± .29
4	2-inch	15.77± .55	11.27± .55	27.03± .60
5	12-inch	15.87± .26	8.72± .38	24.59± .37
6	2-inch	14.93± .55	11.79± .54	26.72± .32
7	6-inch	16.17± .51	9.63± .58	25.80± .84
8	2-inch	16.33± .43	9.27± .58	25.59± .52

In the first picking there is no significant difference between the two blocks of 12-inch spaced plants and any of the three adjacent blocks of 2-inch spaced plants, which was also the case with the two 6-inch spaced blocks in comparison with the four adjacent 2-inch spaced blocks.

Neither is there any significant difference in the yield of the first picking between the four 2-inch blocks, between the two 6-inch blocks, or between the two 12-inch blocks, though the difference in the latter instance is 1.31 ± 0.494 which is almost three times the probable error.

Since there is no significant difference between blocks of like spacing each spacing can be treated as one array, but even with the reduced probable errors secured in this way there is no significant difference between any of the spacings in the yield of the first picking.

In the second picking 2-inch blocks showed increases in yield over adjacent 12-inch blocks of 2.78 ± 0.394, 2.55 ± 0.667, and 3.07 ± 0.66 pounds, all of which are considered significant differences, being 7.06, 3.83, and 4.65 times the probable error respectively.

The increase in yield of 2-inch spaced blocks over adjacent 6-inch blocks of the second picking was not so great as the increase over 12-inch spaced blocks. The differences in favor of the 2-inch are 1.90 ± 0.495, 2.49 ± 0.647, and 2.16 ± 0.792 pounds. The first two differences are to be considered significant as they are 3.84 and 3.85 times the probable error, but the last one in only 2.73 times

the error. In one comparison the 2-inch spaced block gave 0.36 of a pound less than the adjacent 6-inch spaced block, but this reduction is less than the probable error of either of the compared weights. However, as there is no significant difference between any of the blocks of like spacing each spacing can be treated as one array which gives an increase of 2-inch over 12-inch spaced plants of 2.44 ± 0.355 pounds, which is 6.88 times the error, and an increase over 6-inch spaced plants of 1.54 ± 0.344 pounds, which is 4.48 times the error. On this basis the writer finds that 2-inch spaced plants gave a significantly higher yield than 6- or 12-inch spaced plants in the second picking.

The total yields of the 2-inch spaced blocks gave an increase over adjacent 12-inch spaced blocks in every comparison. The increases are 3.39 ± 0.675, 2.44 ± 0.70, 2.13 ± 0.489, which are 5.02, 3.47, and 4.36 times the error and can be considered significant.

Although 2-inch spaced blocks outyielded adjacent 6-inch blocks in all but one case, where the difference was only 0.21 of a pound, these differences can not be considered significant.

Since there is no significant difference in total yield between any of the blocks of like spacing each spacing can be treated as one array, as with the first and second picking. On this basis the 2-inch spaced plants show an increase of 2.77 ± 0.441 pounds over 12-inch spaced plants, which is 6.28 times the error. The increase of 2- over 6-inch spaced blocks, however, is only 0.95 ± 0.5, which should not be considered significant.

The mean yield of the first, second, and total pickings of 123.5 feet of row space is shown for each spacing in Table V.

TABLE V.—*Mean yields of the first, second, and total pickings of 123.5 feet of 2, 6, and 12-inch spaced plants, Coachella Valley, 1923*

Spacing	First picking	Second picking	Total yield
	Pounds	*Pounds*	*Pounds*
2-inch	15.55±0.25	10.75±0.27	26.30±0.24
6-inch	16.14±.29	9.21±.34	25.35±.45
12-inch	15.22±.26	8.31±.23	23.53±.37

The results of the 1923 test not only substantiate the conclusion drawn from the 1922 test, that no reduction in yield occurs when plants are left as close together as 2 inches in the row, but indicate that increased yields may be secured from close-spaced plants. Although the first picking of the 1923 test showed no increase in the yields of the 2-inch spacings over the 6-inch and 12-inch, the 2-inch spacings forged ahead in the second picking, and gave significant increases in yield.

With plants spaced as close as 2 inches in the row there is a tendency to suppression or restriction of the development of the lower fruiting branches, but even in the first picking it is shown that this handicap is made up by the larger numbers of plants, so that the yields were not reduced below that of 6- or 12-inch spaced plants. The same degree of crowding does not continue throughout the season, as weaker plants are overtopped by their more vigorous neighbors, and as they become larger the plants spread apart, so the rows appear wider and the foliage less dense, allowing a full crop of bolls to be set and matured.

Figure 3 shows the block yields of the first and second picking of the different spacings for both the 1922 and 1923 experiments. It can readily be seen that the yields were about the same in the 1922 test, but the 1923 test shows a rather consistent alteration in block yields, the higher yields being coincident with the 2-inch spacing.

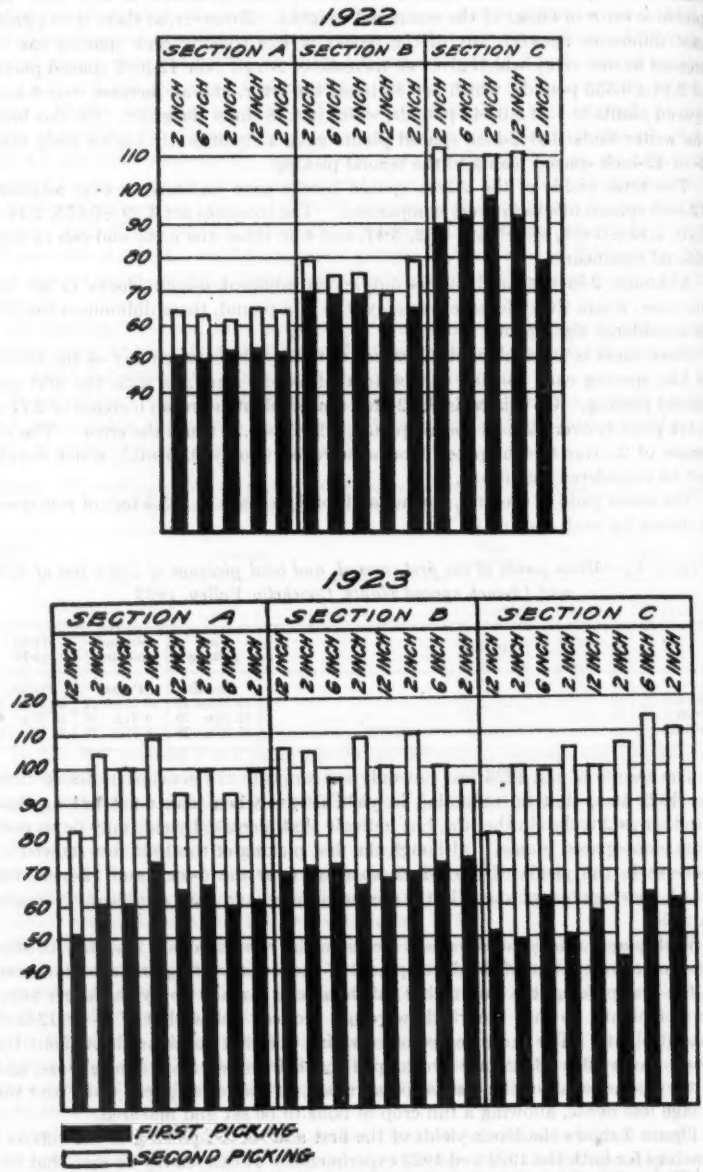

FIG. 3.—Block totals in pounds of spacing experiments, Coachella Valley, Calif., 1922 and 1923

Comparisons were not made between close spacings and large, rank, overgrown plants with many vegetative branches, which would be expected to show a greater advantage for close spacing, so that the present comparison between no thinning or 2-inch spacing with spacings of 6 and 12 inches is believed to be more significant. The 6- and 12-inch spaced plants were not crowded and did not have large numbers of vegetative branches, but an earlier thinning of these blocks would have allowed more vegetative branches to develop, with the result of greater crowding and closing of the lanes between the rows, which undoubtedly tends to reduce the yields and would have given the 2-inch spacing an additional advantage.

While large differences in yield between unthinned and wider-spaced plants in the irrigated valleys of the Southwest are not to be expected under conditions where both spacings are well grown, the ability of the close-spaced plants to set a good crop in a short time may often secure an advantage where the soil is not very well adapted to cotton culture, where the farmer is not familiar with the crop, or where irrigation is inadequate. Even if the greatest care is used in irrigation it is not to be expected that the plants can be kept in the best condition for the production of fruit throughout the entire season. Water is likely to be held off too long occasionally, which causes shedding, or may be applied too frequently, causing overgrowth and consequent shedding. The degree of this kind of damage is in proportion to the skill with which the farmer irrigates his cotton. On this basis it becomes apparent that the ability of the close-spaced plants to set more bolls during short periods of favorable conditions may also be of value in irrigated districts. This does not mean that good yields are not secured from the wide-spaced plants under favorable conditions, but that the chances of a full crop, especially under less favorable conditions apparently are better with close spacings than with wide spacings, even under the long-season conditions of the irrigated valleys.

CONCLUSIONS

Experiments under irrigation in the Coachella Valley of California of plants spaced approximately 2, 6, and 12 inches apart in the row indicate that no reduction in yield occurs when plants are left as close as 2 inches in the row, and that occasionally increased yields may be obtained from 2-inch spaced plants, even under long-season, weevil-free conditions.

It also is indicated that plants spaced at 6 inches give equal or better yields than 12-inch spaced plants. However, since the 6-inch spacing is not convenient in field operations the practical alternatives are 2 plants together at 12 inches or omitting the chopping operation entirely, except in thick stands, when some plants should be pulled out.

In the first picking the yields were nearly the same for all spacings; in the second picking the 6 and 2-inch spacings yielded more than the 12-inch, the greatest increase being in the 2-inch spacing.

Though unthinned cotton, or plants spaced as close as 2 inches in the row, is more crowded at first, which may result in a suppression of some of the lower fruiting branches, as well as of the vegetative branches, the crowding is lessened by the spreading apart of the plants as they become larger. This is in contrast with the behavior of plants that are widely spaced at first, which produce numerous vegetative branches and become more crowded as the season advances.

Since even on the best soil and with the greatest care it is not likely that the plants can be kept in the best condition for the production and retention of fruit throughout the entire season, the ability of the close-spaced plants to set a crop rapidly during favorable periods is likely to prove of advantage even under the long-season, weevil-free conditions of the Southwest.

CORRELATION AMONG QUANTITATIVE CHARACTERS IN MAIZE[1]

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Varieties of maize differ greatly in general size and in the length of time required to produce a crop. As a rule, the large varieties require a longer growing period and produce a greater yield than the small varieties.

The breeder, in producing a type of maize for special conditions, often is confronted with the task of combining the characters of high productivity with earliness and therefore it is desirable to know to what extent the various characters of size and season are correlated among themselves.

If intervarietal correlations were calculated it would be found that the characters of size and season were very closely associated, but such correlations would afford little basis for predicting the facility with which such combinations could be dissolved through hybridization. With many combinations there are, of course, physiological limitations which can not be surmounted, but a consideration of the varieties in existence shows that physiological limitations permit a wide range of combinations.

It has been shown that size characters in maize are inherited and that the mode of inheritance can be explained in accordance with Mendelian principles by hypothecating numerous stable heritable elements or factors.[2] Since these factors are distributed among 10 chromosomes it follows that numerous genetic correlations among size characters are to be expected, but while such correlations increase the difficulty of making new combinations they seldom are insurmountable.

Correlations among various characters of maize have been studied by several investigators, chiefly in searching for a criterion of selection within a variety.[3, 4, 5] Such studies have shown an almost complete independence of the characters of the ear with subsequent yield or other size characters, but since they were made within a single variety in each case they do not afford a secure basis for predicting complete freedom of recombination in hybrids between diverse forms.

MATERIAL

The present investigation was undertaken with a view to measure the correlations among certain quantitative characters in hybrids between varieties of maize offering the maximum size differences.

As the diminutive parent the writer has used the well-known Tom Thumb variety of pop corn which, excluding abnormal dwarf forms, probably is the

[1] Received for publication June 27, 1924—issued November, 1924.

[2] EMERSON, R. A., and EAST, E. M. THE INHERITANCE OF QUANTITATIVE CHARACTERS IN MAIZE. Nebr. Agr. Exp. Sta. Research Bul. 2, 120 p., illus. 1913.

[3] EWING, E. C. CORRELATION OF CHARACTERS IN CORN. N. Y. Cornell Agr. Exp. Sta. Bul. 287, p. 67–100, illus. 1910.

[4] LOVE, H. H. THE RELATION OF CERTAIN EAR CHARACTERS TO YIELD IN CORN. Ann. Rpt. Amer. Breeders' Assoc. 7 : 29–40. 1912.

[5] LOVE H. H., and WENTZ, J. B. CORRELATION BETWEEN EAR CHARACTERS AND YIELD IN CORN. Jour. Amer. Soc. Agron. 9 : 315–322. 1917.

smallest type of normal maize. The gigantic parent used is a variety of maize from the west coast of Mexico, designated Jala.[6] Ears and seeds of both varieties are shown in Plate 1 and the relative differences in size between them are shown diagrammatically in figure 1.

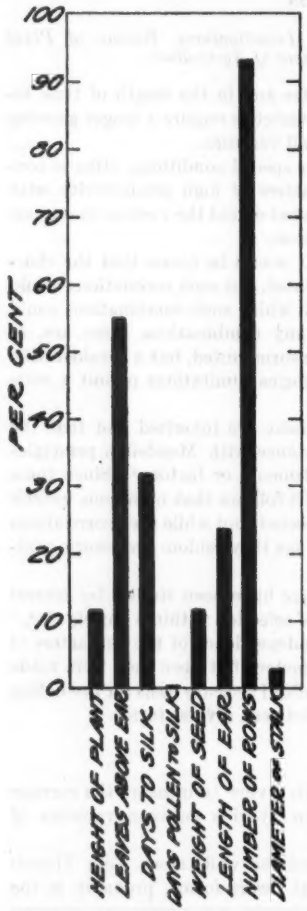


FIG. 1.—Diagrammatic representation of the differences between Jala and Tom Thumb maize. Jala taken as 100

The cross was made with the large variety as the female parent and a small F_1 population was grown at Lanham, Md. From this F_1 population two self-pollinated ears were chosen to provide the F_2 generation. One of these ears was perfectly normal with yellow, light yellow, and white seeds in the proportion of 1–2–1 (100 yellow, 246 light yellow, 119 white) while the other had but two seed classes, yellow and white in approximately equal numbers (170 white, 163 yellow) with a large proportion of deficient seeds consisting of hardly more than a pericarp. Since the Tom Thumb parent has a yellow endosperm and that of the Jala parent is white it was thought possible that the large proportion of deficient seeds represented some incompatibilities in recombinations.

These F_2 populations together with progenies of both parents and the remainder of the F_1 seed were grown at Chula Vista, Calif., where the moderate climate and long growing season permitted the latest segregates and parents to mature normally.

In view of the absence of light yellow seeds on one ear, together with the high proportion of sterile ovularies, the several seed classes were grown separately. This proved to be a needless precaution, as there were no outstanding differences between the two F_2 populations nor in the several seed classes, as is shown by the biometrical constants in Table I. In analyzing the correlations, however, only one F_2 population was used, namely, that not having the complication of deficient seeds. The correlations were calculated separately for each seed class but since there were no significant differences between the classes only the coefficients for the entire population are presented.

[6] KEMPTON, J. H. JALA, A LARGE VARIETY OF CORN FROM MEXICO. Jour. Heredity 15 :337–344. 1924.

INHERITANCE OF THE QUANTITATIVE CHARACTERS

The designations of the characters studied are self-explanatory, with the possible exception of "weight of seed," which is the average weight of a single seed produced by the plants measured, not the weight of the seed planted. The frequency distributions for several of the characters in which the difference between the parents was greatest are shown in figures 2 to 5. These polygons include in

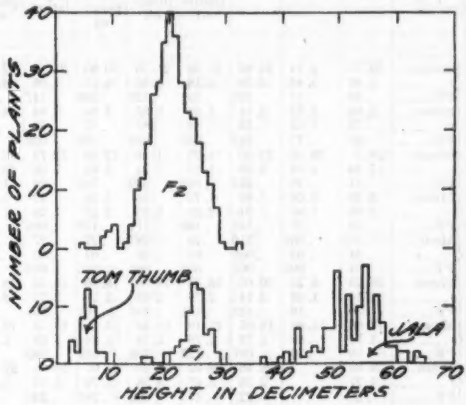


FIG. 2.—Frequency distribution for height of plant. Estimated factorial difference between parents 28

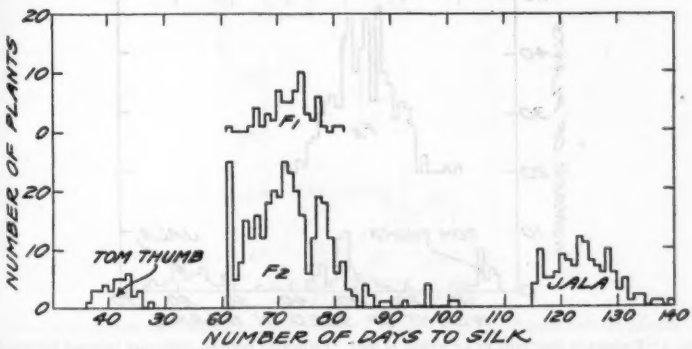


FIG. 3.—Frequency distributions for days to silk. Estimated factorial difference between parents 26

each case the parents, the F_1 and the F_2 generations. In all the characters studied except number of rows on the ear, the F_1 generation may be said to be intermediate between the parents, though actually below the mean of the parents in five cases and above in both the length of ear and in number of rows. In some cases the size of the larger parent is approximated. With number of rows the F_1 exceeds the larger parent, the increased number tending to be maintained in the F_2 remaining above the mean of the parents.

TABLE I.—*Biometrical constants for Jala and Tom Thumb maize and their F_1 and F_2 hybrid populations*

Character designation	Constants	Jala	Tom Thumb	F_1	F_2 with normal seed classes				F_2 with deficient seeds		
					White seeds planted	Yellow seeds planted	Pale yellow seeds planted	Entire population	White seeds planted	Yellow seeds planted	Entire population
Height in dcm.	mean	52.75	6.14	24.80	21.86	21.20	20.80	21.17	22.80	21.10	21.69
	σ	5.06	1.46	2.59	4.16	3.66	4.15	4.06	4.59	3.92	4.50
	PE	.30	.15	.222	.291	.320	.204	.147	.632	.542	.34
Number of leaves above ear.	mean	5.98	3.32	4.41	3.98	3.85	3.94	3.94	4.50	4.48	4.49
	σ	.78	1.63	.53	.84	.87	.72	.76	.64	.91	.79
	PE	.05	.17	.045	.059	.076	.036	.028	.089	.091	.064
Number of days to silk.	Mean	124.7	39.50	73.20	74.22	71.10	72.50	72.73	77.80	74.31	75.52
	σ	7.44	2.52	3.93	5.17	7.34	7.66	7.06	10.17	6.74	8.26
	PE	.44	.28	.342	.360	.639	.385	.26	1.39	.673	.67
Days pollen to silk.	Mean	6.20	0.00	1.91	1.73	.88	1.56	1.49	1.50	2.04	1.86
	σ	2.88	1.89	1.91	2.26	1.33	2.55	2.28	1.68	3.21	2.76
	PE	.18	.23	.167	.160	.114	.128	.084	.231	.322	.226
Weight of a single seed in grams.	Mean	.562	.064	.310	.28	.28	.27	.27	.25	.25	.25
	σ	.34	.02	.030	.05	.03	.04	.05	.07	.04	.05
	PE	.04	.003	.003	.004	.003	.002	.002	.01	.005	.004
Length of ear in cm.	Mean	26.25	6.22	20.00	16.73	15.60	16.50	16.42	18.20	17.79	17.94
	σ	5.54	1.49	2.14	2.79	2.33	3.21	2.73	3.37	2.80	3.01
	PE	.30	.19	.185	.191	.230	.161	.100	.463	.29	.248
Number of rows on ear.	Mean	12.68	11.80	14.05	13.19	13.40	13.13	13.19	13.20	12.80	12.93
	σ	1.74	.69	1.13	1.78	1.24	1.64	1.62	1.45	1.56	1.67
	PE	.10	.10	.097	.122	.108	.083	.060	.199	.16	.139
Diameter of stalk in mm.	Mean	39.05	.....	28.16	26.92	26.60	27.07	26.95	27.00	25.26	25.88
	σ	4.27	.....	2.56	5.47	5.38	5.70	5.50	5.08	4.37	4.60
	PE	.25	.....	.22	.375	.469	.281	.200	.70	.44	.374

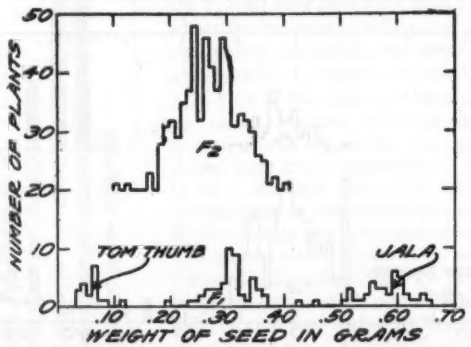


FIG. 4.—Frequency distributions for weight of seed. Estimated factorial difference between parents 19

By use of Castle's formula as improved by Wright [7] for estimating the factorial difference in the case of each character, it is seen that the least number of factors involved for the four characters, days to silk, height of plant, length of ear, and weight of seed, is with the character, length of ear, where the formula indicates a difference of 17–18 factors between the two parents, while 28 factors are indicated for height of plant.

While too much emphasis should not be placed upon this method of estimating the number of heritable factors involved, it is believed that the method underestimates rather than exaggerates the actual number. This would seem to follow

[7] CASTLE, W. E. AN IMPROVED METHOD OF ESTIMATING THE NUMBER OF GENETIC FACTORS CONCERNED IN CASES OF BLENDING INHERITANCE. Science 54: 223. 1921.

from the fact that in maize the occurrence of more than 20 factors almost certainly would necessitate linkages. If the linkage were perfect the effect would be of a single factor and as the degree of linkage decreased the effect of two independent factors would be approached.

With the exception of the number of days to silk, the F_2 distributions are fairly regular and very similar to those for the plants of the F_1. The distribution for number of days to silk suggests trimodality with a large group of early plants and a fairly well-defined population of late plants. However, the parental ranges are not reached and no plants were obtained as early as the latest of the early parent nor as late as the earliest plants of the late parent and the factorial difference between the parents is estimated at 26.

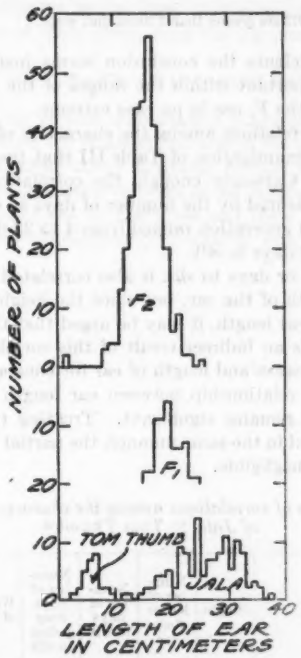


FIG. 5.—Frequency distributions for length of ear. Estimated factorial difference between parents 17-18

In the other characters the parental types were represented in the extreme plants of the F_2 distributions, but the parental ranges were not exceeded except in the previously-mentioned case of number of rows on the ear. No frequency polygon is given for this latter character, since the parental difference was not large.

CORRELATION BETWEEN THE CHARACTERS

The coefficients of correlation among the characters of the F_1 plants may be taken as an indication of the physiological as distinct from the genetic relationships. An examination of the F_1 coefficients given in Table II shows that there is only one significant correlation, namely, that between length of ear and number of rows, indicating that the conditions which favored the production of long ears also resulted in the development of a large number of rows.

TABLE II.—*Coefficients of correlation among the characters of the plants of the F_1 of Jala × Tom Thumb*[a]

Character designation	Height	Leaves above ear	Days to silk	Pollen to silk	Weight of seed	Length of ear	Number of rows	Diameter of stalk
Height		0.26	0.23	0.18	0.12	0.28	0.16	0.14
Leaves above ear	0.26		.13	.05	−.07	.16	.22	.00
Days to silk	.23	.13		.02	−.23	.07	.12	−.05
Pollen to silk	.18	.05	.02		.10	.05	.26	−.00
Weight of seed	.12	−.07	−.23	.10		.37	.11	.03
Length of ear	.28	.16	.07	.05	.37		.43	.11
Number of rows	.16	.22	.12	.26	.11	.43		.07
Diameter of stalk	.14	.00	−.05	−.00	.03	.11	.07	

[a] Coefficients in excess of 0.272 are greater than 3 times their error.

From these F_1 coefficients the conclusion seems justified that physiologica correlations are not important within the ranges of the characters studied, but the ranges involved in the F_1 are in no case extreme.

Proceeding to the correlations among the characters of the second generation, it will be seen from an examination of Table III that the closest correlations are with height of plant. Curiously enough, the correlation of plant height with length of season, as measured by the number of days to silk, is not high, though the plants of the second generation ranged from 4 to 33 decimeters in height and required from 62 to 102 days to silk.

The length of season or days to silk is also correlated with the weight of the seed and with the length of the ear, but since the height of the plant is rather closely correlated with ear length, it may be urged that the relationship between season and ear length is an indirect result of this correlation. Calculating the partial correlation for season and length of ear for constant height of plant, it is found that though the relationship between ear length and season is reduced to .131 the correlation remains significant. Treating the correlation between season and weight of seed in the same manner, the partial correlation for constant height becomes .096 or negligible.

TABLE III.—*Coefficients of correlations among the characters of the plants of the F_2 of Jala × Tom Thumb*[a]

Character designations	Height	Number of leaves above ear	Number of days to silk	Number of days from pollen to silk	Weight of seed	Length of ear	Number of rows	Diameter of stalk
Height		0.107	0.174	0.044	0.350	0.457	0.025	0.568
Number of leaves above ear	0.107		.089	.097	.062	.108	.162	.098
Number of days to silk	.174	.089		.338	.149	.194	−.093	.107
Number of days from pollen to silk	.044	.097	.338		.105	.071	−.027	.065
Weight of seed	.350	.062	.149	.105		.382	−.087	.290
Length of ear	.457	.108	.194	.071	.382		.084	.380
Number of rows	.025	.162	−.093	−.027	−.087	.084		.039
Diameter of stalk	.568	.098	.107	.065	.290	.380	.039	

[a] Coefficients in excess of 0.119 are greater than 3 times their error.

From a consideration of the correlations with days to silk there would seem to be no great obstacle to combining earliness with large ears, heavy seeds, or even tall plants. On the other hand, more difficulty would be encountered in attempting a combination of short plants with long ears and heavy seeds or in producing stocky plants by combining short stature with large diameter.

It seems worthy of note that while the plants of the F_1 showed a close correlation of ear length with number of rows, this correlation shrinks essentially to zero among the plants of the second generation.

Contrary to the conditions found among some animals where the factors affecting size are found to be general for the organism, the lack of correlations among several of the characters studied in this hybrid would seem to indicate a rather high degree of independence of the factors influencing the various parts of the plants.

When the probable number of heritable factors involved in these size characters is taken into consideration, together with the low degree of correlation, it seems certain that the distribution of these factors is fairly general among the 10 chromosomes, lending support to the view that in maize the chromosomes are more or less duplicates of each other, each having complementary factors for most of the characters.

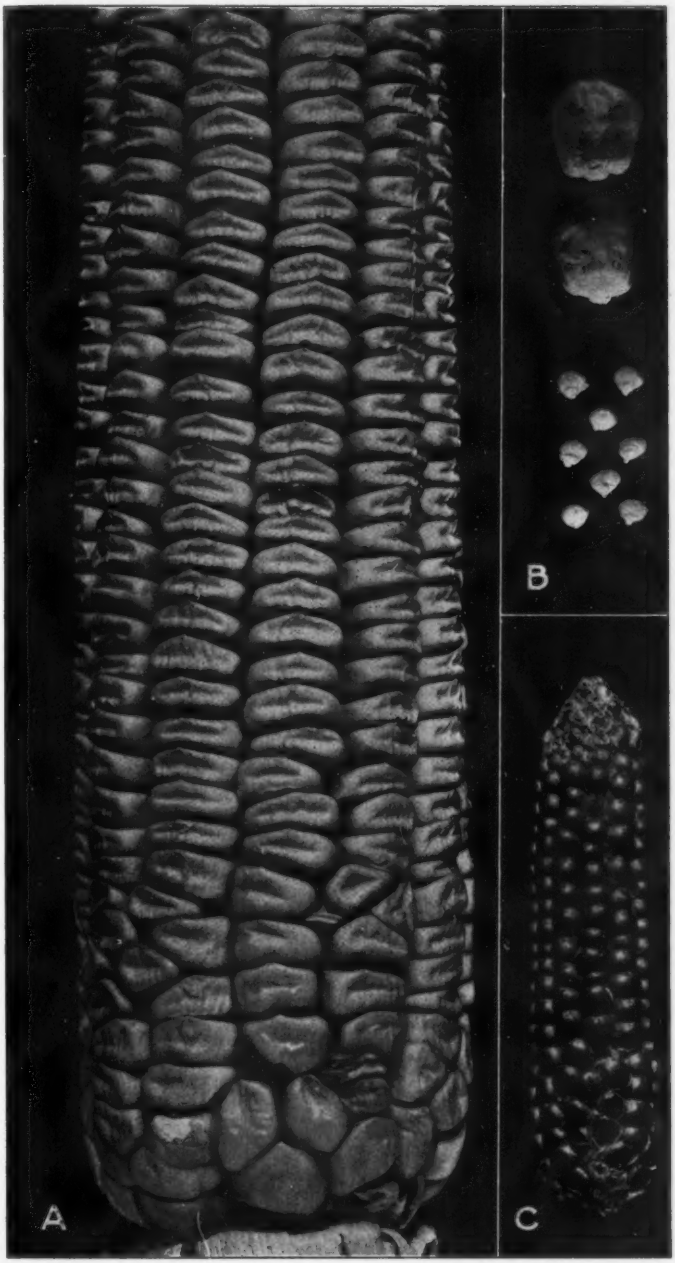
SUMMARY AND CONCLUSION

The correlations among certain quantitative characters of maize have been studied in a hybrid between the smallest and one of the largest varieties. While apparently there are a great many heritable factors involved in the several differences between large and small varieties of maize, the correlations among the characters of size often are low or negligible and there seems to be no genetic obstacle to forming recombinations of desirable quantitative characters by hybridization.

PLATE 1

Section of an ear of Jala maize, an ear of the Tom Thumb variety, and seeds of both showing differences in size. (Natural size.)

(1102)


A
B
C

EFFECT OF FUMIGATION UPON HEATING OF GRAIN CAUSED BY INSECTS[1]

By E. A. Back, *Entomologist in Charge*, and R. T. Cotton, *Entomologist, Stored Product Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

The heating of grain in storage is one of the serious problems confronting the farmers and grain dealers. A moisture content in wheat of 14.5 per cent has been fixed by the Bureau of Agricultural Economics of this department as a line between safety and possible danger, so far as the moisture content affects the keeping quality of wheat in storage. It is well known that the heating of grain may be caused by a high moisture content, and grain dealers have been put to much expense in devising methods of quickly detecting unusual rises of temperature in stored grain (by means of electric thermometers, for instance), and in preventing its continuance by transferring from bin to bin, by mixing lots of grain of different moisture content, or by passing the grain through drying machines. With heating due to causes other than insect infestation, this paper does not deal.

From the foregoing statements it may be assumed that when wheat shows a moisture content of less than 14.5 per cent, it is likely to remain in condition if given proper storage. Grain dealers, farmers, and seedsmen are aware, however, that seeds containing a lower percentage of moisture may heat badly if they become infested with insects. Thus, wheat arriving at the Baltimore grain terminals during 1923 was found heating as a result of infestation by the Angoumois grain moth[2] when its moisture content was but 12 per cent. In farmers' bins wheat is very often found heating as a result of attack by the rice[3] and granary weevils,[4] while beans, cowpeas, and chick-peas are commonly infested by bruchids, and heat badly as a result.

During the season of 1919–20, G. H. Baston, of the then Bureau of Markets and Crop Estimates of this department, recorded (*15*)[5] observations made during the storage at Buffalo, N. Y., of 15,000,000 bushels of wheat belonging to the war-time Grain Corporation. In spite of the fact that this wheat had an average moisture content ranging from 11.3 to 12.6 per cent, it was found during the winter months that it was in part going out of condition, and it became necessary to run the bins to determine the actual state of the grain. Nine hundred and seventy-seven bins were transferred and examinations made; of these, 374 were in good condition, 396 were "found to contain a trace of weevil and beetle infestation,"[6] and 207 were found "infested with weevil and beetle." By taking

[1] Received for publication Apr. 22, 1924—issued November, 1924.

[2] *Sitotroga cerealella.*

[3] *Sitophilus oryza.*

[4] *Sitophilus granarius.*

[5] Reference is made by number (italic) to "Literature cited," p. 1116.

[6] By "trace of weevil and beetle" it is meant that specimens of insects were found among the kernels which were not apparently damaged, although they may have contained the immature stages; the term "weevils" refers primarily to the rice weevil, *Sitophilus oryza*, and to a lesser extent to the granary weevil, *Sitophilus granarius;* the term "beetle" refers to the flour beetle, *Tribolium confusum*, to the flat grain beetle, *Cryptolestes pusillus*, and to the saw-toothed grain beetle, *Oryzaephilus surinamensis*, and possibly to several others of the smaller grain beetles.

Journal of Agricultural Research, Washington, D. C.

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Key No. K-143

samples of grain for moisture analyses, and taking temperature readings at the time the grain was run from bin to bin, Mr. Baston gathered the data presented in Table I.

From these data he concludes that the temperature was highest in the bins which were infested, that the moisture content was lower in the bins which showed a trace of infestation or a pronounced infestation with weevil, and that these facts indicate that the moisture was not the factor that caused the rise in temperature. "Eliminating the factors of moisture, damage, and time in storage, one by one," the data indicate "plainly," so he states, "that the rising temperature was caused by the weevil and the beetle." These conclusions are shown diagrammatically in figure 1, the left-hand end of which represents the conditions of temperature and moisture for the uninfested grain, the right-hand end those for the highly infested grain, and the center those for that with a trace of infestation.

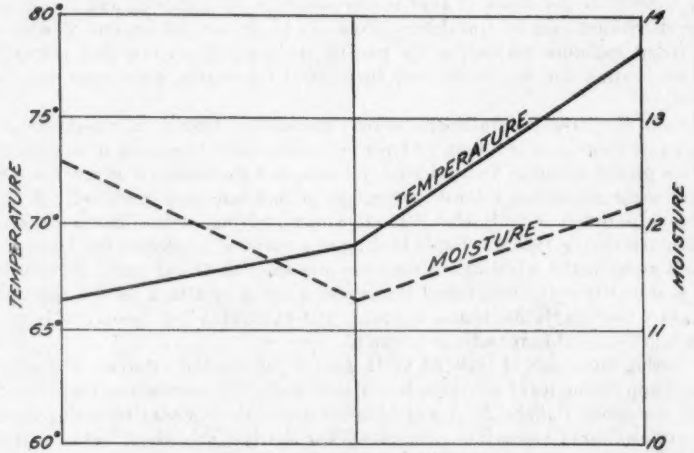

FIG. 1.—Diagram showing average condition of 977 bins of wheat handled at Buffalo, N. Y., in 1919-20

TABLE I.—*Correlation of general condition of wheat in 977 bins, showing average moisture, average temperature, and other data, for good, slightly infested, and highly infested wheat*

General condition	Average moisture content	Damaged wheat (average)	Time in storage	Number of bins of wheat	Percentage of bins of wheat	Average temperature
		Per cent	*Days*			°*F.*
Good. Free of insects	12.6	3.1	105	374	38.3	66.2
Showing trace of insects	11.3	2.9	118	396	40.5	69.3
Highly infested	12.2	2.9	90	207	21.2	78.3

With these conclusions, entomologists who have had experience with seed-infesting insects can readily agree. Considering the numerous instances of heating seeds with which the writers have come in contact during the past few years, it seems strange that in 1892 Doran should state (*6*, *7*) that he knew of

but three previously published records in American entomological literature containing reference to high temperatures caused by grain-infesting insects.

Dr. L. O. Howard, in 1888, records (*3*) the sending to the then Division of Entomology by Judge Lawrence Johnson at Holly Springs, Miss., of seeds of *Dolichos* species badly infested with *Bruchus scutellaris*. These seeds had been stored in a paper sack of a gallon capacity, filling it about one-third full. With the temperature of the air surrounding the bag standing at 71° F., a thermometer placed in the seeds recorded 96° F. The difference of 25° F. was thought to be due in great part to a mechanical cause—the gnawing of the peas by the beetles and their larvae.

W. D. Richardson, of Frederick, Va., in a letter of November 1, 1891, addressed to Dr. E. A. Schwarz and published in "Insect Life" (*5*), states that on August 13, he had occasion to move a bag of peas which had stood in a corner of a room in his house for a month or more, and from which thousands of *Bruchus scutellaris* had been emerging. On touching the bag he was surprised at the temperature, and discovered that while the temperature of the room was 70° F., that within the sack of peas registered 88° F. This temperature continued for about two weeks, when it commenced to fall and the beetles soon thereafter ceased to emerge. Prof. A. J. Cook stated in 1889 (*4*) that insects had been known to produce heat in grain, but gave no temperature records.

Doran states (*7*) that in February, 1892, at the Maryland Agricultural College his attention was called to a bin of bran or middlings 12 feet long by 6 feet wide, which was badly infested with *Silvanus* (*Carthartus*) *cassiae*. He discovered that, with the room temperature at 53° F., the temperature of the bran taken about 1½ feet below the surface ranged from 42° to 95° F. These extremes of 42° and 95° F. were obtained from spots in the bin not more than 3 feet apart. The temperature of 95° F. was confined to a small space not more than 2 to 3 feet in diameter, and within this the beetles and their larvae were very numerous. He states that the bran was "perfectly dry with no indication of mold or ferment." The lower temperature of 42° F., or near that, was found in various places in the same bin and in the bins and sacks of bran stored in the same room. Doran took a sample of bran from the spot showing the highest temperature and found 1 pound of it to contain about 1,500 beetles. There were 103 weevils in an ounce of bran, which gave an estimate of 1,648 beetles to the pound. As 100 of these beetles weighed 23 grains, those in a pound of bran were estimated to weigh about 345 or 350 grains, or about one-twentieth of the entire weight of the bran and insects combined.

Lintner in 1893 wrote (*8*) that wheat in Montgomery County, Pa., in 1892, was so badly infested with the Angoumois grain moth that when sacked directly from the threshing machine it would heat overnight. In 1901, Dr. L. O. Howard stated (*9*) that this pest was so abundant in the 1900 crop in Pennsylvania, New Jersey, and Delaware wheat that the grain heated badly. Dr. F. H. Chittenden observed (*10*) that heating may be caused independently of the attack of insects, but an abundance of insects in stored cereals frequently causes such heating that the temperature of infested material may be increased 25° to 40° F. above the temperature of the surrounding air. In 1915 Newell in Texas wrote (*12*) of the heavy infestation of sudan grass seed by the Angoumois grain moth, the peculiarity of the infestation being the resulting heating of the infested seed. Back (*16*) in 1922 gave data on the heating of chick-peas infested with the four-spotted bean weevil. Heating of wheat as a result of infestation by *Sitotroga cerealella*, *Sitophilus oryza*, *S. granarius*, *Tribolium confusum* and *Cryptolestes pusillus* was noted by the writers as a common occurrence in farmers' bins in Montgomery County, Md., during the winters of 1921–22 and 1922–23.

The heating of grain caused by insect infestation was known many years previous to American observations. It should be recorded that in 1762 Duhamel du Monceau and Tillet, in discussing the Angoumois grain moth wrote (*1*):

> The emergence of moths is usually preceded by a heating which spreads in the sheaves and piles of grain. Thermometers register 25° R. (89° F.) to 30° R. (100° F.) when the exterior air is only 15° R. (66.2° F.). The cold of autumn stops development and no more moths are seen until spring. Heat in the grain must be generated by the insects assembled in great numbers. Grain heavily infested becomes very warm. No heat was noticed in slightly infested grain, and the heat disappeared when most of the moths had emerged. Sometimes the heat passes promptly, at others it lasts for 3 or 4 weeks. It may result from a fermentation caused by the humidity that insects produce. It is certain that when the harvest is wet and warm rains follow, the piles of grain heat in a very short time and the insects breed rapidly. Without doubt, the heat is very favorable to the development of the moth, and the damp grain is more easily attacked by the insects. In 1760, when it was hot and dry, the people did not expect any insects, but they were very abundant, although the wheat did not heat noticeably, until mid-September. In 1761 the grain was very hot, its temperature being 53° R. (about 150° F.). Each year, even when harvests are dry, wheat heats independently of insects. However, the heat is always greater in infested wheat than in clean wheat, so that we can attribute it partly to the insects and partly to the humidity. When humidity causes an increased heat, everything is more favorable for the multiplication of the insects and when this multiplication takes place, the heat increases still more.[1]

The immediate interest of the writers has been centered in the possibility of reducing the temperature of heating grain to normal. In the references already quoted regarding the development of heat in grain due to insects, no mention is made of preventing the heating by remedial measures directed against the insects themselves, except that Back states (*16, p. 23–24*) that fumigation with hydrocyanic-acid gas kills *Bruchus quadrimaculatus*, "reduces the temperature of chick-peas to normal and stops spread." However, Herpin (*2, p. 9*) in 1860 stated that hot wheat infested with the Angoumois grain moth cools off in a few days after the insects are killed by asphyxiation, and this seems to be the only reference definitely answering the query "Will fumigation bring back to normal the temperature of grain which has heated as a result of insect attack?" The writers wish to record several opportunities given them to make observations upon this point.

During the summer of 1918, because of the congested warehousing conditions in New York City, one hundred and thirty-seven thousand 240-pound sacks of chick-peas, grown in Mexico, were held in six large warehouses in New Orleans. In October of that year the owners discovered that many sacks were heating badly because of infestation by *Bruchus quadrimaculatus*. Opportunity was given the writers to become personally familiar with the problem as presented in the various warehouses. Because of the demand for space resulting from war conditions, the sacks of seed had in many instances been stacked 20 deep. Heating sacks were found in all warehouses, and as the approaching cold of winter had no effect upon the heating sacks, it was recommended that the warehouses be fumigated with hydrocyanic-acid gas. In one tier of sacks temperature readings were made with ordinary soil thermometers on November 30 and December 1, with the results indicated in figure 2.

The temperature of the warehouse at the time these readings were made was 58° F. Uninfested sacks registered rather uniformly 60° F., while as noted in Figure 2, the temperature of the infested sacks ranged as high as 103° F. On December 1 the warehouse was fumigated with hydrocyanic-acid gas, using 2½ pounds of sodium cyanid per thousand cubic feet of space, this being the dosage desired by the owners. One week later, after the warehouse had been thoroughly ventilated and opened throughout the day for five days, readings were again made with soil thermometers. The warehouse temperature outside the sacks was 68° F. at 11 a. m. The temperatures secured for sacks numbered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, and 29, were 64°, 65°, 66°, 64°, 68°, 66°, 72°, 65°, 72°, 67.5°, 68°, 67°, 68°, 67°,

[1] Condensed translation, with temperatures in Fahrenheit added.

66°, 72°, 69°, 68°, 68°, 64°, 68°, 74°, 68°, 68°, 64.3°, and 70° F., respectively. While the warehouse temperature stood at 68° F. when these readings were in progress, the temperature of the warehouse over a 24-hour period was undoubtedly somewhat lower, and it was the belief of the writers that the temperature of the sacks was still falling slowly to about 60° F. No opportunity for further readings was given, and no record was made of the moisture content of the seed. The fumigations, conducted similarly in all six warehouses, was thoroughly effective and in each warehouse the heating in the sacks disappeared after the fumigations. This was naturally a matter of great concern to the owners, since the retail value of the seeds at this time was about $5,000,000.

Studies were made during December, 1921, and January, 1922, of certain bins of wheat on farms near Washington. These bins were infested by the Angoumois grain moth, the rice weevil, and the flat grain beetle, the last two predominating. On December 21, when the barn temperature registered 27° F., the temperature of the wheat in one bin about 15 by 5 by 4 feet was 58° F. at its surface, 104° F. 6 inches below the surface, and 109° F. 2 feet below the surface. The upper layer of wheat for several inches from the surface was caked and mouldy. This wheat was fumigated with carbon disulphid on December 21 and left well covered with a tarpaulin until December 23, when the

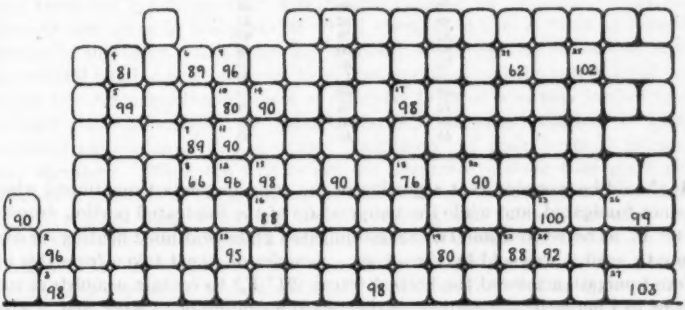

FIG. 2.—Diagram showing ends of 240-pound sacks of chick-peas arranged in single tier, with temperature in degrees Fahrenheit, the heat being due to infestation by *Bruchus quadrimaculatus*

tarpaulin was removed. Examination on December 23 showed the temperature at the bottom of the bin to be 76° F.; 2 feet from the surface at end of bin, 78° F.; and 2 feet from the surface in the center, 100° F. The tarpaulin appeared to be responsible for the slow fall in temperature up to December 23. On December 31 readings were taken which showed the temperature in the center at the ends to be 60° F., and in the middle of the bin 54° F. Final readings taken on January 4, 1922, gave the temperature as 40° F. for both the center and the ends of the bin.

In a second bin about 18 by 18 feet, in which the grain was not over 2 to 3 feet deep, the temperature due to the rice weevil and the flat grain beetle gave readings of 98°, 98°, 93°, 71°, 94°, 98°, 90°, 100°, 38°, 38°, and 38° F., respectively, according to the spot chosen for the reading. These temperatures were attained in wheat from 1 to 1½ feet below the surface at a time when the outside temperature was 30° to 40° F. In a third bin, where the wheat was spread to a depth of from 1 to 1½ feet on a floor beneath which was an open wagon shed there were spots of rice weevil infestation where the temperature ranged as high as 92° F. Other temperatures recorded for such shallow grain were 76°, 88°, 82°, 91°, 90°, 70°, 74°, 78°, and 45° F. Both of these last-mentioned bins were fumigated with carbon disulphid on January 11, 1922. Temperature readings were made on January 13 and January 20, with the results recorded in Table II.

TABLE II.—*Fall in temperature of grain following fumigation with carbon disulphid*

BIN No 1

Temperatures before fumigation, January 11, 1922	Temperatures, January 13	Temperatures, January 20
°F.	°F.	°F.
100	60	40
90	50	40
98	56	40
94	56	40
71	50	40
93	50	40
98	56	40
98	58	40

BIN No. 2

°F.	°F.	°F.
76	60	40
88	62	40
82	58	40
91	60	40
90	60	40
70	55	40
92	58	40
74	54	40
78	58	40
45	40	40

It should be recorded that a portion of the grain in the last-mentioned wheat was not fumigated, and while the temperature of the fumigated portion dropped to 40° F., as noted in Table II, the unfumigated grain continued heating for over a month until it was sold by the owner. Samples of wheat taken from this bin before fumigation showed the hottest wheat (91° F.) to contain a moisture content of 15.1 per cent; the cold, unheating wheat a content of 13.9 per cent. After fumigation, the grain which had been hottest gave upon analyses a moisture content of 14.4 per cent.

CAUSE OF HEATING IN GRAIN

Factors causing heating of grain are not well understood. Enzymatic action and growth of molds such as *Aspergillus* are said to be associated with increasing temperature when no insects are present. Bailey and Gurjar state (*13*) in the conclusions to their report that the deductions from their investigations "support the findings of earlier investigations that spontaneous heating in damp grain is occasioned by the biological oxidation of dextrose and similar sugars, chiefly in the germ or embryo of the kernel." Dendy and Elkington (*14*) in 1919 published an article on the prevention of heating in wheat by means of air-tight storage, basing their work upon the assumption that the heating of the grain is confined to those portions to which air has free access, and not taking insects into consideration. The temperature of about 150° F. (53° R.) recalled by Duhamel du Monceau and Tillet in 1762 in their report on the Angoumois grain moth must have been caused by agencies other than insects, for grain pests are killed by temperatures above 125° F. It is believed by some that the heating of grain is sometimes started by insects and that their development makes it possible for other agencies not so well understood to gain a foothold and carry on the destruction. In discussing a paper by Doran (*7*), presented before the Washington Entomological Society in 1892, its author expressed the opinion that heating of

grain was due to the conversion by the insect at the time of transformation of a "certain amount of so-called 'latent heat' into sensible heat." In other words, that a certain amount of "heat energy" is expended in the operation. This seems especially probable owing to the more or less complete histolysis during the pupa stage. Dr. C. L. Marlatt (*7*), present at the meeting, was of the opinion that the heat was partly produced by fermentation. Austin (*7*) said that he thought the heat due to the chemical action of the forces setting up the fermentation with the grain, while Dr. E. A. Schwarz said (*7*) that the accumulation in numbers of insects produced considerable heat, and referred to the hibernation in crowds of *Megilla maculata*.

The heat produced by the honeybee cluster in winter has been well studied by Phillips and Demuth (*11*), who state that as the temperature of the air in the hive falls in winter the bees become less active until a certain critical temperature (about 57.2° F.) is reached, at which the bees undertake by muscular activity, not unlike that of shivering, to produce heat in order to keep warm. By such action the honeybees are able to maintain a temperature within their cluster of 86° to 95° F., when the outside temperature is 32° F.

The writers have been told by owners of grain elevators that in transferring grain from one bin to another, a transaction known as "running," they have observed hot spots in the grain at which there would be a "ball of insects." Because of the value of the grain, the necessity for prompt action on the part of the owner if heating grain is to be kept from spoiling, and the reticence of most owners to confide specific instances of difficulty to those who may inadvertently transmit trade information to competitors, it is extremely difficult to gain a first-hand knowledge of this so-called "balling-up" of grain pests at points in large elevators. While the writers are not prepared to state that grain pests do make a practice of congregating in cold weather to develop a temperature making possible their continued activity throughout the cold months, they believe that this does happen at times and that this phenomenon offers an interesting field for investigation. The coming together of large numbers of adult insects, as in the case of the bran in which Doran recorded finding approximately 1,500 beetles to the pound, might afford reason for believing that adult insects only are capable of starting an increase in the temperature of grain. Without denying entirely that adults alone are capable of initiating the heating process in grain, the writers are confident that in many instances the developing larvae are quite as important, if not more so.

That the immature stages of *Bruchus quadrimaculatus* must play a large part in the heating of chick-peas would appear from the data of Tables III and IV, in which is recorded the infestation of six samples of 50 seeds each, taken from sack No. 25 of figure 2, which at the time the samples were taken had in the center at 1 foot from the end a temperature of 102° F. These 300 seeds bore 2,174 unhatched eggs, 527 larvæ less than half grown, 453 larvæ one-half to full grown, 98 pupæ, 193 adults about to emerge, and showed indications of 98 adults already emerged (though not necessarily living) at the time of examination. Assuming that each sample of 50 seeds weighed 1 ounce (as one average sample actually did), sack No. 25 contained an infestation of 3,550,960 bruchids in various stages of development. Likewise, sack No. 7 (figure 2) with a temperature of 89° F., if it had an infestation indicated by the data in Table III, might have an estimated infestation of 1,286,640 *Bruchus quadrimaculatus* in all stages.

A study of the more detailed data on the infestation of Sack No. 25, as set forth in Table IV, will give the reader a very concise idea of the degree of infestation by *Bruchus quadrimaculatus*. No such complete data on infestation in relationship to heating of wheat as those here given covering the infestation of chick-peas had ever been presented. The writers present in Tables V and VI, as a report of progress, the rather meager data which they have.

TABLE III.—*Data on the infestation of chick-peas by Bruchus quadrimaculatus*

Sack number	Temperature of seeds	Sample No.	Number of seeds examined	Number of seeds infested	Number of Bruchus—									
					Eggs	Emergence holes	Larvæ				Pupæ	Adults in seeds	Total larvæ, pupæ, and adults	Eggs unhatched
							Eighth grown	Quarter grown	Half grown	Full grown				
25	102° F.	1	50	47	540	11	16	6	19	77	31	51	200	
		2	50	47	506	13	8	13	33	53	26	43	176	
		3	50	49	619	22	97	14	35	39	10	25	220	
		4	50	48	523	17	59	26	16	52	11	30	194	
		5	50	48	548	24	24	11	35	55	19	37	182	
		6	50	49	706	11	174	79	30	8	1	7	299	
Totals			300	288	3,543	98	378	149	168	285	98	193	1,271	2,174
7	80° F.	1	50	50	308	32	2	6	30	111	70	41	260	
		2	50	49	208	32	3	0	8	93	60	32	196	
		3	50	47	262	32	8	1	5	85	43	25	167	
		4	50	45	175	37	6	0	7	58	48	17	136	
		5	50	50	296	23	3	1	19	100	49	11	183	
		6	50	47	286	6	22	15	31	63	20	4	155	
Totals			300	288	1,625	162	44	23	100	510	290	130	1,097	366

TABLE IV.—*Infestation of 50 seeds from each of six samples [a] taken with grain-car probe from a 240-pound sack of chick-peas infested with Bruchus quadrimaculatus and registering 102° F.*

Seed No.	Adults unemerged	Pupæ	Larvæ				Total number of insects in seed	Emergence holes
			Full-grown	Half-grown	Quarter-grown	Eighth grown		
SAMPLE NO. 1								
1	1					2	3	1
2	5						5	2
3	1						1	2
4	1	2	2		1		6	2
5					1	2	3	
6	1		1				2	
7	2	3	3				8	2
8	2		2	3			7	1
9	2		2			3	7	
10	1		1				2	1
11	1	1	1				3	
12	1	1	6			2	10	
13	1	3	3				7	
14			3		1		4	
15	2	1	2				5	
16	1	1					2	
17			3	1			4	
18	2	2		1			5	
19						2	2	
20			3				3	
21	2	1					3	
22			2		2		4	
23			2	1			3	
24	3	1	3	1			8	2
25						4	4	
26		1					1	
27	3			1			4	1
28			2	1			3	
29				3			3	
30			2		1		3	
31	2						2	
SAMPLE NO. 1—Continued								
32		1					1	3
33	2	1	2				5	1
34	2	2	5	2			11	2
35	4		1				5	1
36						2	2	
37	1						1	1
38	1	4	6				11	1
39	2	1	3				6	
40							0	
41	1	1	4				6	
42	1	2	5	2			10	
43	1		3	3			7	3
44		2	1				3	
45							0	
46	1						1	
47							0	
48	1						1	2
49	4		1				5	1
50	2	1	6	4			13	
SAMPLE NO. 2								
1	2		2	1			5	
2	2	1	2				5	
3				2	1		3	
4	1		2		2		5	
5	1	2	4				7	
6	1		2				3	
7		1				2	3	

[a] Samples 1 and 6 being at the extreme ends of the sack; samples 3 and 4 being nearest the center; all samples taken, naturally, along the central longitudinal axis of the sack.

TABLE IV.—*Infestation of 50 seeds from each of six samples taken with grain-car probe from a 240-pound sack of chick-peas infested with Bruchus quadrimaculatus and registering 102° F.*—Continued

Seed No.	Adults unemerged	Pupæ	Larvæ: Full-grown	Larvæ: Half-grown	Larvæ: Quarter-grown	Larvæ: Eighth grown	Total number of insects in seed	Emergence holes
SAMPLE NO. 2—Continued								
8			2	3			5	
9	2		4				6	1
10	2		2	1			5	1
11	2	1			2		5	1
12		1		1			2	1
13	1						1	1
14	1						1	
15		5	2				7	1
16	1						1	1
17	1						1	1
18	2	2	3		3		10	1
19	3						3	1
20	1						1	
21			3				3	
22				3	1		4	
23	1	1	3				5	
24			1				1	
25	1			4			5	
26							0	
27		1					1	
28				3			3	
29	2		3	5			10	1
30						2	2	
31						1	1	
32	2	2					4	
33	1		1	1			3	
34			2	2			4	
35	2	3					5	
36	1						1	
37				1			1	
38	1		1		1		3	
39	1	1	2				4	1
40			1	2			3	
41		1			2	1	4	
42		1	2				3	
43	2		1				3	
44						2	2	
45	2		2				4	
46							0	
47	1			2			3	
48	2	1	3	3			9	
49	1	1	2				4	
50	1	1	1				3	
SAMPLE NO. 3								
1	1					2	3	
2					3		3	1
3			2		1		3	1
4	1	1	2				4	3
5	1		1				2	
6	1	1	1				3	1
7	1	1	1				3	
8			1			1	2	
9				1		2	3	
10						2	2	
11	2						2	1
12	1					1	2	
13					2	1	3	1
14					1	4	5	
15					2	6	8	
16						6	6	
17						5	5	
18			2	8			10	
19		1	2	3			6	
20	1	1	2			5	9	
21	1		1	3			5	
22	2	2	4				8	2
23			1	3			4	

Seed No.	Adults unemerged	Pupæ	Larvæ: Full-grown	Larvæ: Half-grown	Larvæ: Quarter-grown	Larvæ: Eighth grown	Total number of insects in seed	Emergence holes
SAMPLE NO. 3—Continued								
24				6			6	
25	1					5	6	
26						4	4	
27						5	5	
28						4	4	1
29						4	4	
30				4		2	6	
31	1		2	1			4	
32			3		2		5	
33	2		1	2			5	
34	1					8	9	
35						5	5	
36						4	4	
37			1	2			3	
38						5	5	
39					2	1	3	
40	1					2	3	
41	3		1	1			5	
42	1	1	2				4	1
43			1			3	4	
44	1					2	3	
45				1	1		2	
46	1		2				3	1
47	1		1				2	
48	1		1				2	
49							0	
50		2	6			3	11	
SAMPLE NO. 4								
1	2		2	2			6	
2			2		2		4	
3	1		3			2	6	
4	2			3			5	
5	3		1				4	
6	1		2			3	6	
7			2			3	5	
8			3		2		5	
9						1	1	
10						1	1	
11			3		7		10	
12						4	4	
13						3	3	
14					3		3	
15						5	5	
16						3	3	
17						3	3	
18			1	3			4	
19	2	1	3				6	
20			2	2			4	
21						3	3	
22						3	3	
23						4	4	
24					2	1	3	
25			1	1			2	
26						3	3	
27	3		4				7	1
28		2	1				3	
29	3	1	2	1			7	1
30	1	2	1				3	1
31				2	1		3	
32				1	2		3	
33	3	1					4	1
34							0	
35					2	2	4	
36	2	1	2				5	1
37	2		1	1			4	
38			2	1			3	
39			3	3			6	

TABLE IV.—*Infestation of 50 seeds from each of six samples taken with grain-car probe from a 240-pound sack of chick-peas infested with Bruchus quadrimaculatus and registering 102° F.*—Continued

Seed No.	Adults unemerged	Pupæ	Larvæ: Full-grown	Larvæ: Half-grown	Larvæ: Quarter-grown	Larvæ: Eighth grown	Total number of insects in seed	Emergence holes
SAMPLE NO. 4—Continued								
40						1	1	
41						2	2	
42		1					1	
43	1					3	4	
44						2	2	
45			1	1			2	
46	3	2	7				12	
47							0	
48			3				3	
49						4	4	
50	1						1	1
SAMPLE NO. 5								
1					1	2	3	
2	2		3				5	
3			2		3		5	
4	1	1				3	5	
5	2	1	1	4			8	1
6	3	2	3				8	
7		2	3	1			6	
8	3		2				5	
9		1	2	1			4	
10				3			3	
11	2	3	6				11	
12			2	1			3	
13				3			3	
14	2			3			5	
15			1	2			3	
16		1	1	1			3	
17	2	3	2		3		10	
18	1					1	2	
19	1		2	2			5	
20						6	6	
21		1	1		2		4	
22							0	
23	1						1	
24							0	
25						1	1	
26	4		2				6	
27		1	1				2	3
28	2		4				6	1
29			1	1			2	1
30	2						2	1
31			4	2			6	
32	2	1		5			8	
33							0	
34	1				2		3	
35						4	4	
36	1					2	3	
37	1		2				3	1
38			2	1			3	
39	2						2	1
40		1	4	1			6	1
41	1		1	1			3	1
42	1	1	4	2			8	
43		1		4			5	
44						2	2	
45				2		1	3	

Seed No.	Adults unemerged	Pupæ	Larvæ: Full-grown	Larvæ: Half-grown	Larvæ: Quarter-grown	Larvæ: Eighth grown	Total number of insects in seed	Emergence holes
SAMPLE NO. 5—Continued								
46						2	2	
47		1	4				5	1
48	1		4				5	
49	1	2	3				6	
50	1	4	3				8	
SAMPLE NO. 6								
1						8	8	
2	1		2	2		2	7	
3				2		4	6	
4				2		5	7	
5	2			1		1	4	
6	1					4	5	
7	2					5	7	
8						2	2	
9						6	6	
10						2	2	
11				2		3	5	
12						6	6	
13						3	3	
14	1			3		3	7	
15						3	3	
16				3	2	3	8	
17				3	3	2	8	
18					4	5	9	
19					4	2	6	
20					6	6	12	
21					5	5	10	
22				3		2	5	
23			1			8	9	
24		1				5	6	
25					3	4	7	1
26						6	6	
27				1		4	5	
28			1		4		5	
29						3	3	
30					8		8	
31						6	6	
32					9		9	
33					4	1	5	
34	1		1	8			10	
35					2	8	10	
36						13	13	
37			1	2		9	12	
38					8		8	
39						4	4	
40						9	9	
41						8	8	
42						3	3	
43							0	
44					4		4	
45					5		5	
46					4		4	
47						2	2	
48			1	1	2		4	
49					2		2	
50					1	7	8	

The data of Table V on the relative abundance of the rice weevil and the flat grain beetle in probes taken at varying points in a wheat bin having warm spots indicate, as one might expect, a large number of insects present in the warm spots. The data are unsatisfactory in that there are not enough records of the abundance of insects in grain having temperatures below 70° F., for at temperatures between 70° and 90° F. grain pests are thoroughly active.

TABLE V.—*Number of Sitophilus oryza and Cryptolestes pusillus found in one-ounce samples taken at four depths in bin with varying temperatures* [a]

Point in bin	Temperature at center	Number of insects in sample from—								Total insects for probe
		Bottom of bin		1 foot from bottom of bin		1½ feet from bottom of bin		6 inches from top of grain		
		Sitophilus oryza	*Cryptolestes pusillus*	*Sitophilus oryza*	*Cryptolestes pusillus*	*Sitophilus oryza*	*Cryptolestes pusillus*	*Sitophilus oryza*	*Cryptolestes pusillus*	
	°F.									
A	38	1	0	0	0	0	0	1	0	2
B	38	0	1	3	0	1	3	0	0	8
C	38	0	0	1	2	0	1	0	0	4
D	100	1	2	14	12	17	15	4	2	67
E	90	6	18	15	35	14	22	6	10	126
F	98	0	0	2	6	10	30	6	8	62
G	94	6	12	5	5	3	13	10	20	74
H	71	8	5	18	14	21	5	7	3	81
I	93	3	4	8	13	16	27	6	2	79
J	98	6	11	6	9	9	9	8	0	58
K	98	0	4	4	5	17	16	9	0	55

[a] Reading taken where wheat was about 2½ feet deep.

The data of Table VI are of importance in that they give one an idea of the high percentage of wheat kernels actually found infested when examined for immature stages beneath the compound microscope.

The writers can not help but conclude from their observations that the insects themselves are capable of developing heat in grain in storage. It does not seem probable that there is much truth in the often expressed idea that the heating of insect-infested grain is usually due to a fermentation set up by the absorption of moisture by the fæces of the insect. The writers have seen seeds (wheat, beans, and chick-peas) badly damaged and heating because of insects, that were leathery rather than brittle because of the moisture apparently absorbed by the seeds, or the faeces in the seeds. Thus badly infested seeds in sack 25 of Table III could be pressed flat without breaking because, as the writers thought, of the humid conditions produced by the infestation. Yet the fact remains that the heat left not only this sack, but all the sacks of chick-peas fumigated with hydrocyanic-acid gas in the warehouses, and the wheat fumigated with carbon disulphid in farmers' bins. It may be recalled, also, that Richardson (*5*) and Duhamel du Monceau and Tillet (*1*) have said that the heating of seeds ceases often with the passing of the infestation (usually after the seeds have been rendered valueless as food and filled with excreta). Unless it can be proved that bacteria or other organisms causing heat are killed by fumigation with hydrocyanic-acid gas or carbon disulphid, there seems no other conclusion than that the activity of certain stored-product pests is directly responsible for the development of heat in grain in certain instances and that fumigation will kill such heat and permit the grain to return to normal temperature.

TABLE VI.—*Percentage of wheat kernels infested and number of adult insects found free in ounce samples in bin the wheat of which registered 58° F. at surface, 104° F. 6 inches below surface, 109° F. 2 feet below surface, and 88° F. at bottom of bin about 4 feet below surface*

Depth at which sample was taken	Location of probe											
	At end of bin				At center of bin				At end of bin			
	Per cent of kernels infested	Number of specimens of—			Per cent of infested kernels	Number of specimens of—			Per cent of kernels infested	Number of specimens of—		
		Oryza	Pusillus	Parasites		Oryza	Pusillus	Parasites		Oryza	Pusillus	Parasites
6 inches	92.1	2	6	3	93.2	2	4	2	88.9	6	3	3
12 inches	94.3	27	12	0	94	7	14	8	93.9	7	7	3
18 inches	90.9	7	12	3	96.4	7	15	3	90	6	8	3
24 inches	92.3	3	17	13	93.7	4	4	6	96.5	3	3	0
30 inches	91.8	4	9	3	91.2	4	3	3	92.3	3	5	0
36 inches	93.4	3	5	1	90	1	4	2	84.2	3	3	6
42 inches	95.5	24	6	6	91.4	2	10	2	89.8	4	10	9
48 inches	90	4	1	0	91.1	1	7	2	94.9	32	14	9

DESTRUCTION OF HEAT BY FUMIGATION OF MOST PRACTICAL VALUE

Whatever may be the reader's conclusion regarding the actual cause of heating in insect-infested seeds, there can be no doubt as to the value of any treatment that will reduce the temperature of the seeds to normal. The placing of heating seeds in cold storage will not prevent insect activity if the seeds are sacked or placed in bulk and are already heating when placed in cold storage. Cowpeas infested with *Bruchus quadrimaculatus* and beans infested with *Bruchus obtectus* continued to heat for several months when placed in cold storage at 32° F., according to the experience of several operators of storage plants.

In ordinary storage where seeds are stacked as indicated in figure 2, the heating seeds radiate sufficient heat to raise the temperature appreciably in the spaces between the sacks. Thus, with a warehouse temperature of 58° F. the space between sacks No. 22, 23, and 24 (sacks with temperatures of 88°, 100°, and 92° F., respectively) registered 78° F. In many instances the temperature in the spaces between or touching heating sacks was raised to 70° and 72° F. Bruchids at 58° F. are not capable of mating or spreading, but at temperatures above 70° F. they reproduce actively and bring about a spreading of the infestation at a season of the year when the owner normally expects protection from insects due to the action of cold weather. The same holds true in wheat bins on the farm, even in bins of wheat where the seeds are not deeper than 1 to 2 feet. The heating permits feeding and reproduction to progress even in the dead of winter. A thorough fumigation with hydrocyanic-acid gas or carbon disulphid kills the insects or a sufficient number of them so that those that remain can not maintain a condition favorable to the production of progressive destruction.

SUMMARY

It is well known that the heating of grain may be caused by a high moisture content. Spontaneous heating in damp grain is occasioned by the biological oxidation of dextrose and similar sugars, chiefly in the germ or embryo of the kernel. Enzymatic action and growth of molds have been said to be associated with increasing temperatures when no insects are present. With heating due to causes other than insect infestation this paper does not deal.

It has been assumed by certain grain authorities that when wheat shows a moisture content of less than 14.5 per cent it is likely to remain in condition if

given proper storage and kept free of insects. However, when insects are present grain has been known to heat when the moisture content is as low as 12 per cent. A study of 15,000,000 bushels of wheat by the Bureau of Markets indicated that when the moisture content was as low as 11.3 per cent to 12 per cent heating due to insect infestation occurred, and resulted in the conclusion, by eliminating factors of moisture, damage, and time in storage, that the rising temperatures were caused by insect infestation.

The heating of grain and grain products when insects are present has been noted before. The writers have known chick-peas in 240-pound sacks to heat as a result of attack by *Bruchus quadrimaculatus*, the temperature rising to as high as 103° F. when the normal temperature was 58° F. Wheat stored in farmers' bins and well infested by *Sitophilus oryza* and *Cryptolestes pusillus* was found to have developed a temperature as high as 109° F. when the normal temperature was 27° F. Wheat in shallow bins or on barn floors and stored in piles ranging in depth from 1 to 3 feet was found at times heating if badly infested by the two pests just mentioned and the Angoumois grain moth. It is not difficult to find many instances of heating in cereals and such cereal products as animal feeds.

The cause of heating when insects are present in numbers is not clearly understood. Probably the most prevalent opinion is that heating is due to fermentation started by the attraction of moisture to the fæces of the insects. Others have given as the cause the mechanical friction due to the insects' feeding. It is known that the honeybee cluster in winter can develop by muscular activity not unlike shivering a temperature in the hive of 86°–95° F. when the normal temperature is but 32° F. Grain dealers claim that insects do congregate in large numbers in spots in grain in elevators and that such "balls of insects" are responsible for starting grain heating. As many as 1,500 *Carthartus cassiae* beetles have been recorded in 1 pound of bran heating at a temperature of 95° F. The writers estimated one 240-pound sack of chick-peas with a temperature of 102° F. to contain 3,550,960 Bruchids in various stages of development; the sacks not heating, and having a normal temperature of 58° F., showed no infestation. It is undoubtedly true that when grain heats badly as a result of insect infestation the insects are present in great abundance.

The fumigation of grain, heating as a result of insect infestation, with either carbon disulphid or hydrocyanic-acid gas brings about a fall in the temperature of grain to normal. This was found true in the fumigation of one hundred and forty thousand 240-pound sacks of chick-peas stored in six warehouses and containing hundreds of heating sacks. It was also found true in the fumigation of heating wheat in farmers' bins.

The destruction of heat in grain during winter is of great importance, particularly on the farm. In ordinary storage heating seeds radiate sufficient heat to raise appreciably the temperature in the spaces between the sacks in the case of sacked seeds. The temperature, which was 58° F. between uninfested sacks of chick-peas, was raised to as high as 70° to 78° F. between infested and heating sacks, with the result that the Bruchids, dormant at 58° F., were able to multiply and actively spread the infestation from the infested and heating sacks. The activity of pests in stored corn and wheat is in like manner made possible by heating. Heating permits feeding and reproduction to progress even in the dead of winter. Fumigation does what cold weather will not do; it kills the heat and reduces the temperature of the seeds to normal and thus prevents progressive destruction.

Unless it can be proved that bacteria, molds, or other agencies causing heat are killed by fumigation with hydrocyanic-acid gas or carbon disulphid, the writers conclude that the activity of certain stored-product pests is directly responsible for the development of heat in grain in certain instances and that fumigation will kill such heat and permit the grain to return to normal temperature.

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THE NITRIFICATION OF PHOSPHORUS NITRIDE [1]

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Phosphorus nitride has recently received some attention as a possible fertilizer product from the fixation of atmospheric nitrogen. A survey of the literature furnished no direct information as to the fertilizer value of this material. As a result of vegetation experiments, Stutzer [2] found that silicon nitride and other nitrides were of little or no value as fertilizers. The article does not state whether phosphorus nitride was included in this work. In order to secure definite information on phosphorus nitride, the experiment reported below was made. This work was based upon the well-known fact that materials which do not nitrify readily do not serve as satisfactory nitrogenous fertilizers, although in unusual cases it is possible that such materials may have some plant food value.

The phosphorus nitride used in this study was prepared by the action of ammonia on phosphorus pentasulfide and contained 40.03 per cent nitrogen. The purity of the sample on the basis of the formula P_3N_5 was, therefore, 93.3 per cent. It was shown that the sample contained an excess of phosphorus and it is probable that this element accounts for the greater part of the remaining 6.7 per cent. It was probably present largely as free phosphorus, but some compounds of the type PNS and PNO may have been present also.

The experiment was made with a Susquehanna loam soil, using 100-gm. samples in 300-cc. beakers. In order to provide optimum conditions for nitrification, 0.5-gm. calcium carbonate was added to each soil sample. The phosphorus nitride and the ammonium sulphate used for comparison, as well as the calcium carbonate, were thoroughly mixed with the dry soil and 21 per cent distilled water added. The soil samples were maintained at room temperature during the course of the experiment and any water lost by evaporation was restored frequently. At intervals, nitrate determinations were made on a group of samples, using the phenoldisulphonic acid method. These results are given in Table I.

[1] Received for publication Apr. 28, 1924—issued November, 1924.

[2] STUTZER, A. UEBER NITRIDE IN IHREN WIRKUNGEN AUF PFLANZEN. (Abstract) Chem. Ztg. 34: 1015. 1910.

TABLE I.—*Comparison of nitrate formation in soils receiving phosphorus nitride and ammonium sulphate*

Treatment	Mgm. N added per 100 gm. of soil	Nitrate nitrogen	Average	Increase over control	Nitrate nitrogen	Average	Increase over control
		19 days			35 days		
		Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
Control	0	6.58 7.14	6.86	----------	7.44 7.88	7.66	----------
Ammonium sulphate	5	10.00 10.42	10.21	3.35	11.33 11.84	11.59	3.93
Do	10	10.00 10.00	10.00	3.14	16.54 17.37	16.96	9.30
Do	20	9.62 9.26	9.44	2.58	26.05 26.72	26.39	18.73
Phosphorus nitride	5	7.58 7.35	7.47	.61	8.40 8.40	8.40	.74
Do	10	7.14 7.58	7.36	.50	8.14 8.14	8.14	.48
Do	20	7.35 7.35	7.35	.49	8.14 8.02	8.08	.42
		56 days			75 days		
Control	0	8.68 9.30	8.99	----------	8.40 8.68	8.54	----------
Ammonium sulphate	5	14.89 14.08	14.48	5.49	14.08 13.36	13.72	5.18
Do	10	18.61 19.30	18.96	9.97	18.61 19.30	18.96	10.42
Do	20	28.94 27.42	28.18	19.19	28.16 27.42	27.79	19.25
Phosphorus nitride	5	8.98 8.40	8.69	−.30	8.40 8.54	8.47	−.07
Do	10	8.98 8.68	8.83	−.16	8.54 8.68	8.61	.07
Do	20	8.98 8.98	8.98	−.01	7.89 8.14	8.02	−.54

It will be observed from the table that the addition of phosphorus nitride to soil, maintained under optimum conditions for nitrification, did not markedly affect the nitrate content. During the first month there was a slight increase but later a decrease in nitrates. This initial slight increase may have resulted from the nitrification of the phosphorus nitride or, on the other hand, this material may have merely stimulated the nitrification of the soil organic matter. It is impossible to state which of the two explanations is the correct one. However, it is certain, judging from the limited amount of data here presented, that the nitrogen in phosphorus nitride is not readily converted into nitrates and since plants for the most part require nitrate nitrogen for their best growth, it is very improbable that phosphorus nitride would supply that demand.

AECIAL STAGES OF THE LEAF RUSTS OF RYE, PUCCINIA DISPERSA ERIKSS. AND HENN., AND OF BARLEY, P. ANOMALA ROSTR., IN THE UNITED STATES[1]

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INTRODUCTION

In 1918, investigations on the leaf rusts of rye, barley, wheat, corn, and related grasses were begun by this laboratory in cooperation with the Office of Cereal Investigations, United States Department of Agriculture. Since it has been shown by Stakman (*11*)[2] and others that, as in northern Europe, *Puccinia graminis* Pers., the stem rust of wheat, oats, barley, rye, and related grasses, can be controlled in the northern United States by eradicating its alternate host, the common barberry, *Berberis vulgaris* L., it seemed important to determine the aecial hosts of the leaf rusts in this country and the rôle they may perform in propagating these rusts. In a previous paper, the writers (*5*) already have reported on the alternate host of the leaf rust of wheat, *P. triticina* Erikss., and at this time are presenting the results of similar studies on the leaf rusts of rye and barley.

LEAF RUST OF RYE, PUCCINIA DISPERSA

DeBary (*3*) established the fact that *Anchusa arvensis* and *A. officinalis* are aecial hosts of *Puccinia dispersa*. This relationship was determined by inoculating the leaves of Anchusa with the basidiospores of the rust fungus. The resulting infection produced aeciospores which, when applied to the leaves of rye plants, produced infection and subsequent development of urediniospores.

When basidiospores of rye leaf rust were sown on *Berberis vulgaris* L., *Rhamnus frangula* L., *R. cathartica* L., *Ranunculus acris* L., *Ranunculus bulbosus* L., *Leontodon taraxacum* L. (*Taraxacum officinale* Weber), and *Urtica dioica* L., no infection resulted. Nielson (*9*) later obtained infection on rye following inoculation with aeciospores from *Anchusa officinalis*. Plowright (*10*) also obtained aecia on *Anchusa arvensis* by placing rusted rye straw near that host. Eriksson (*4*) was able to infect *Anchusa arvensis* and *A. officinalis* with *Puccinia dispersa* on rye, but obtained no infection on *Myosotis alpestris* F. W. Schmidt, *Symphytum asperum* Lepechin (*S. asperrimum* Donn), and *Pulmonaria officinalis* L. and only pycnia on *Nonnea rosea* Link. Sowings of aeciospores from Anchusa produced uredinia on rye but not on the other inoculated grass hosts. Klebahn (*6*) reports on similar experiments in which aecia were obtained on Anchusa. Uredinia also were obtained on rye when inoculated with aeciospores from Anchusa. In the

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[2] Reference is made by number (italic) to "Literature cited," p. 1126.

United States, Arthur (*1*, *2*) secured infection on *Lycopsis arvensis* L. (*Anchusa arvensis*) with *Puccinia dispersa* from rye. A few pycnia developed in one instance and numerous pycnia and a few aecia in another. He states that—

> It is possible, yet scarcely probable, that the explanation (of this weak development of aecia) lies in the lessened vigor of the American rye rust, which is propagated by repeating spores alone, the aecial hosts being practically wanting in America.

In Europe, the leaf rust of rye is characterized by several workers as having teliospores which develop and germinate during the same season. Eriksson (*4*) especially has emphasized this point and states that in Sweden the teliospores begin to germinate as soon as they mature, that is, about the middle of July, and cease germinating when kept out of doors during the winter. The aecia were produced from the beginning of August to the end of September. Although in Eriksson's experiments the teliospores did not germinate after overwintering in the open, he found they would remain dormant until spring if kept indoors. Most of the other European writers on rusts appear to agree with these statements of Eriksson. DeBary (*3*), however, who originally discovered the connection, states that the teliospores germinated after overwintering. Klebahn (*6*) questions whether this was out of doors, pointing out that DeBary does not state the manner in which they were overwintered. DeBary (*3*), however, does state that aecia are to be found throughout the year from spring to late summer and in mild winters even in January.

GERMINATION OF THE TELIOSPORES

During the winter of 1918–19, a number of collections of telia of the leaf rust of rye were wintered along with those of the leaf rust of wheat and were tested for germination in the spring of 1919. One collection of the leaf rust of rye germinated in April. Since, as stated above, the teliospores of the leaf rust of rye are generally believed to germinate only in the late summer and fall of the year in which they are formed, all the collections of telia of this rust, made in the spring and summer of 1919, were tested for germination beginning July 15, and at intervals of two to four weeks until April 7, 1920.

Each collection was tied in a coarse cheesecloth bag and hung on the east side of a building about 2 feet from the ground All the material was brought into the greenhouse two days before testing and placed on moist sphagnum for that length of time. The test for germination was made by scraping some of the teliospores from the rye leaves and placing them in a hanging drop on the bottom of an inverted Syracuse watch glass. These drops were then examined after 12 hours.

Twenty collections of teliospores obtained from the States of Indiana, Georgia, Delaware, New York, Wisconsin, Minnesota, Nebraska, Kansas, Washington, Oregon, and California were thus wintered and tested. Of these, 15 showed germination at some time during the test. Germination first took place on August 19 in 2 collections, and between that date and November 25, 10 additional collections showed germination. All these collections and 2 others continued, for the most part, to germinate during December. During January, 11 of the 14 still showed some germination. In February there apparently was a sharp falling off in the number of collections showing germination, only six showing germination during that period. In March, another collection was added to the list for the first time. On April 7, when the material was last tested, five cultures still showed germination. One of these five was the collection which germinated on August 19 of the preceding summer. This culture showed germination on 9 consecutive occasions during the winter, another 10, a third 7, a fourth 6, and the other, which first showed germination in April, germinated twice.

These results are the more striking when one considers that the teliospores were subjected to sudden and extreme temperature changes, from low winter temperature to the warm temperature of the greenhouse. They then were placed out of doors and again suddenly subjected to low temperatures and the drying effects of freezing. Such treatment certainly would be expected to be fatal to any except the most hardy spores. This may explain why some of the collections did cease to germinate before spring. From the information now available, it would appear that in this country the teliospores of the leaf rust of rye, as a general rule, are capable of germinating whenever conditions are favorable either in the fall or, surviving the winter, in the spring, or both. The repeated germination of some of the collections would indicate that differences existed among the teliospores as to dormancy, otherwise one would expect all the spores to germinate when germination was first observed.

It is difficult to draw conclusions with reference to a rust of this type in which the telia long remain covered by the epidermis, as the length of time the epidermis remains over the telium may prove to be a factor. It is well known, however, that in some species of rusts, especially short-cycled species of Puccinia, there are to be found in the same telium spores which germinate at once and others which germinate only after a considerable resting period.

What the significance of the difference in action of the collections may be is difficult to say. While most of the collections which germinated in the fall continued to germinate until well into the winter, one showed germination only on September 12, and, although tested 13 times during the winter and spring, gave no more evidences of germination. Four others germinated only up to January 5. On the other hand, one collection gave no germination until March 8, although tested previously 10 times throughout the fall and winter. It is possible that in this country the leaf rust of rye may be made up of different strains differing, among other things, in the length of time which it takes to mature their teliospores and in the ability of these to overwinter. On the other hand, these differences may be due largely to environmental conditions under which the spores developed. These are questions which are difficult to solve as the leaf rust of rye, unlike some of the leaf rusts of native grasses, does not form its teliospores upon seedlings, and the time and care which would be necessary to grow different pure cultures of leaf rust of rye on maturing plants under controlled conditions has made it thus far impracticable to attempt to investigate this phase.

The writers' observations to the effect that all the teliospores of *Puccinia dispersa* necessarily do not germinate in the late summer and fall, but some at least may remain dormant during the winter, support the original statements of DeBary (*3*) as to the germination of the teliospores of this rust after overwintering, which have been somewhat questioned. It always has been difficult to determine how the aecial infection occurring in the fall can figure importantly in the winter survival of the rust. Certainly if the rust should not hibernate in the uredinial stage on the rye plant itself, the autumnal aecia can not be a factor in its survival. On the other hand, under such conditions, those teliospores which remain dormant until the next spring may be of importance in regions where the rust does not overwinter on rye itself and the aecial host is present.

From still another point of view, it is interesting to note the germinative ability of the teliospores of *Puccinia dispersa* subsequent to their winter survival. Eriksson was influenced to separate the leaf rust of wheat as a species, *Puccinia triticina*, from the leaf rust of rye largely because the former, unlike the latter, was unable to infect Anchusa and because the teliospores of the leaf rust of wheat germinated only after overwintering, while those of rye germinated shortly after harvest and did not overwinter. Here it may be pointed out that during the

winter of 1919–20, the authors observed that a number of collections of teliospores of the leaf rust of wheat were germinable in early December and continued to germinate until spring in a manner similar to those of *P. dispersa*. The distinction between these species on the basis of difference in time of germination of their teliospores therefore is not as sharp as it first appeared.

Klebahn (*7*) has shown that teliospores which usually are known to germinate only after the close of the winter season may be made to germinate by alternate drying and wetting. Recently, Maneval (*8*) also has shown for a number of such rusts that the teliospores will germinate if alternately wet and dried or soaked long enough. However, it probably is true that the teliospores of *P. triticina* generally germinate only after hibernation while those of *P. dispersa* usually are capable of germinating in the fall of the same year in which they are formed. However, they may retain this ability throughout the winter. Varying climatic conditions in different years, however, may tend to level such a distinction.

INFECTION OF ANCHUSA

The above collections, on being found germinable, were sown on potted plants of *Anchusa officinalis* grown in the greenhouse from seed obtained from Prof. Ed. Fisher of Berne, Switzerland. Of the 15 collections germinating only 5, however, produced infection. Of these 5, only 2 produced both pycnia and aecia, while the other 3 developed pycnia only. Aeciospores from the 2 plants showing aecia were sown on seedling plants of Rosen rye, producing uredinia typical of *Puccinia dispersa*. The 5 collections which gave infection were obtained from Mt. Vernon, Ind., Madison, Wis., Manhattan, Kans., Kearney, Nebr., and Seattle, Wash. These were the same collections which still showed germinating teliospores on April 7. Each of these collections, however, was sown a number of times without obtaining infection. This was especially true during the fall and winter, most of the infections being obtained in the spring, as happened in both instances of aecial production. Apparently the reason for this was the condition of the Anchusa plants, for they developed little new growth in the greenhouse during the fall and winter, and many of the older leaves died. With the advent of spring, new growth took place and more abundant infection resulted. In consequence, no very definite conclusions can be drawn from the negative results obtained from the other 10 collections as they germinated only at times when the Anchusa plants were in poor condition. It is evident, however, that the teliospores of *P. dispersa* which overwintered were able to infect Anchusa, thus establishing their identity with the species *Puccinia dispersa* as distinguished by Eriksson. But for this fact, the overwintering of the teliospores in the absence of infection of Anchusa would have indicated the possibility of their having belonged to another race or species of leaf rust on rye. By the close of winter, such a small amount of material was left that no attempt was made to inoculate plants of Anchusa in the field.

Although infection was not obtained to any extent in the greenhouse in the fall, mulching of Anchusa plants in the field, where they were in good growing condition, resulted in abundant production of aecia. On August 12, 1922, rye straw from the Agronomy plats, heavily covered with telia of the leaf rust of rye, was used to mulch vigorously growing plants of *Anchusa officinalis*. The summer of 1922 was very dry and no rain fell until August 23. On August 31, numerous yellowish spots containing pycnia were noted scattered over the leaves, petioles, and young flower parts. Aecia appeared in these areas in considerable abundance in about a week (Pl. 1, A). Again, on September 22, shortly following a rain, new infected areas containing pycnia were found scattered over the plants. Aecia also followed in these areas. With the fall rains, infection was more or less

continuous. On November 10, when last observed, the plants were covered with a heavy development of aecia and, even then, many newly-formed pycnia were evident. Although the rusted straw remained as a mulch throughout the winter, and the Anchusa plants produced vigorous growth the following spring, no infection was observed. In the fall of 1923, the same plants again were mulched with rye straw laden with teliospores of *Puccinia dispersa*. No infection resulted. This would indicate that, if its aecial host be present, the leaf rust of rye occasionally may produce its aecia in abundance in this country in late summer and fall just as it does in Europe. Also the indications are that the absence of its aecial host apparently has not destroyed in any way the inherent ability of the rust to produce aecia.

At the time *Anchusa officinalis* was mulched with rye straw, other boraginaceous species in close proximity also were mulched with the same material in order to determine whether the rust might not have still other aecial hosts. These Boraginaceae were *Nonnea rosea* Link, *N. lutea* DC., *Cerinthe minor* L., *C. major* L., *C. alpina* Kit., *Lappula echinata* Gilib. (*Myosotis lappula* L.), *Myosotis palustris* Hill, *M. arvensis* (L.) Hill, *M. alpestris* F. W. Schmidt, *M. virginica* (L.) BSP., *Symphytum asperum* Lepechin (*S. asperrimum* Donn) and *Pulmonaria officinalis* L. All these plants were exposed to infection equally with the plants of *Anchusa officinalis*, but in only one case was there any indication of infection. One plant of *Nonnea rosea* developed a few pycnia but the infection proceeded no further. This additional link in evidence points to the identity of the leaf rust of rye in this country and in Europe, for Eriksson (*4*) in Sweden found that *Myosotis alpestris* and *Symphytum asperrimum* were immune, while pycnia developed on *Nonnea rosea*.

Earlier in July, 1920, a new host for the leaf rust of rye was discovered in *Anchusa capensis* Thunb. This had been planted as a border plant around flower beds near the horticultural greenhouses of Purdue University. The plants showed a fairly heavy development of aecia (Pl. 1, B) the spores of which, when sown on rye, produced typical uredinia of the leaf rust of rye. As this sporulation occurred in advance of the application of the straw mulch, the infection must have started from a small plat of rye to the north, or perhaps from straw contained in the manure used in fertilizing the bed. This seems to be the only record of the occurrence of aecia of the leaf rust of rye in this country except where Anchusa plants had been planted and mulched for the purpose of obtaining the aecia. Flowering specimens of this species of Anchusa were sent to Prof. B. L. Robinson of the Gray Herbarium, Harvard University, who kindly had them compared with an isotype of *Anchusa capensis* and reported that they appeared to be identical.

It is not probable, however, that the aecial hosts of the leaf rust of rye are of much importance in this country at the present time. The aecia, with one exception, as stated above, have only been found where Anchusa plants have been mulched with rye straw for the purpose of producing the aecia. *Lycopsis arvensis*, presumably the *Anchusa arvensis* of northern Europe, occurs in North America from Nova Scotia to Ontario, Minnesota, Pennsylvania, and Virginia. So far as we have been able to determine, aecia of *Puccinia dispersa* have never been collected on this species. Since *Puccinia dispersa* overwinters fairly commonly in the uredinial stage on rye, the rust maintains itself without the aecial stage.

It hardly would appear probable that, were the development of the aecial stage in this country to occur in the fall, as it does in Europe, conditions affecting maintenance of the rust would change to any extent. The rust would have to survive the condition either in the aecial stage on Anchusa or, what is more probable, pass

over to rye and survive in the uredinial stage on that host. As volunteer rye in the eastern United States usually is heavily rusted in the fall, in the absence of Anchusa it seems improbable that the presence of the aecial stage would influence the situation to any extent. If the teliospores commonly retain their germinability until spring, Anchusa species may be of some importance in that they may serve as additional centers for spread of the rust. Our observations are not extensive enough to warrant drawing any conclusions as to this possibility, the general situation in Europe indicating its rare occurrence in the spring, even there.

LEAF RUST OF BARLEY, PUCCINIA ANOMALA

The aecial stage of the leaf rust of barley, *Puccinia anomala* Rostr. (*P. simplex* [Körn.] Erikss. and Henn.), was unknown until Tranzschel (*12*), working in Russia in 1914, sowed teliospores on plants of *Ornithogalum umbellatum* L., *O. narbonense* L., *Muscari botryoides* (L.) Mill., *M. tenuiflorum* Tausch, *Scilla sibirica* Andr., and *Allium angulosum* L. Many aecia developed from this sowing on *Ornithogalum umbellatum* and a smaller number on *O. narbonense*. The other plants remained uninfected. Aeciospores from *O. umbellatum* were sown upon *Hordeum vulgare* L., producing urediniospores and teliospores. We have not been able to find records to show that these results have been repeated elsewhere.

Barley straw, heavily laden with teliospores of the leaf rust of barley, was collected at Washington, D. C., Blacksburg, Va., and Mt. Vernon, Wash., in the summer of 1921. Part of each of these collections was wintered in a similar manner to that described for rye, and part was used to mulch small areas where bulbs of the Star-of-Bethlehem, *Ornithogalum umbellatum*, had been planted. The collections of telia, when brought into the greenhouse on March 24, 1922, were found to germinate and were sown on Star-of-Bethlehem plants growing in pots. Infection was obtained in each case, pycnia appearing April 4, followed by aecia April 18 (Pl. 1, C). From these cultures, aeciospores were sown on barley, producing uredinia typical of *Puccinia anomala*. The groups of plants of Star-of-Bethlehem, mulched in the field with straw of each of these collections, also showed infection, pycnia appearing about April 15, followed by aecia. On May 18, uredinia developed on barley sown near the aecia-bearing Ornithogalum plants.

During the summer of 1922, barley straw bearing telia of *Puccinia anomala* was collected at Lafayette, Ind., and used to mulch *Ornithogalum umbellatum* in the field. On April 17, 1923, pycnia were noted upon these plants, followed on May 5 by aecia.

Puccinia anomala more nearly resembles a Uromyces than a species of Puccinia. The teliospores are for the most part one-celled. Indeed, in some collections considerable search is necessary in order to find the two-celled spores. According to usage, however, the presence of these few two-celled teliospores places the species in the genus Puccinia. A very similar rust, *Uromyces hordei* Tracy, is found in southwestern United States upon a wild barley, *Hordeum pusillum* Nutt. This, however, produces only one-celled teliospores, but is otherwise very similar. Arthur (*2*) has shown that this rust has its aecial stage on *Northoscordium bivalve* (L.) Britton, a species very similar and fairly closely related to *Ornithogalum umbellatum*. Although Arthur was not able to infect Ornithogalum with *Uromyces hordei*, the evident relationship of the two rusts invited further study. In March and April, 1920, a telial collection of *Uromyces hordei*, sent by R. S. Kirby from Norman, Oklahoma, was used four times in an effort to infect plants of *Northoscordium bivalve* and *Ornithogalum umbellatum*.

Abundant infection was obtained upon Northoscordium (Pl. 1, D) while no signs of infection were detected on Ornithogalum. Aeciospores from the infection on Northoscordium were sown two different times on seedlings of *Hordeum pusillum* and of common barley, *Hordeum vulgare*, which is susceptible to *Puccinia anomala*. Infection resulted only on *Hordeum pusillum*. Telia obtained from these cultures were overwintered and again sown upon *Northoscordium bivalve* and *Ornithogalum umbellatum* in March, 1921. Infection developed only on the Northoscordium. In addition, five different uredinial cultures of *Puccinia anomala* from different localities, while infecting barley varieties heavily, produced no infection when sown on *Hordeum pusillum*. It seems evident, therefore, that although *Uromyces hordei* and *Puccinia anomala* are somewhat closely related, they are distinctly different as to host specialization, in both the aecial and telial stages.

These results show that *Ornithogalum umbellatum* is an aecial host for the leaf rust of barley in this country as well as in Russia. The four widely separated localities from which the telial collections were made also would indicate that this is likely to be the case for this rust generally over the entire country. The writers have no evidence at the present time to show that the aecial stage is naturally produced, as aecia on this host have never been collected or reported in this country. However, it is entirely possible that they may be produced but have been overlooked, attention not having been directed to this plant, as the rôle of Star-of-Bethlehem as an aecial host was discovered quite recently and apparently is not generally known.

Star-of-Bethlehem may become, if it already is not, of considerable importance in barley-growing areas because of its tendency to escape from cultivation and become a weed. In some places, notably in the Southern States, this plant has become a pest almost equal in importance to wild onion (garlic), as far as occupying cultivated land is concerned. It is a bulbous plant, coming up in the early spring and dying down in midsummer. It multiplies rapidly by division of the bulb and may be scattered widely over the field in plowing and cultivating. It is obvious, therefore, that its presence in barley-growing areas is very undesirable.

Unlike the leaf rust of rye, the leaf rust of barley has not been shown to overwinter in the uredinial stage in this country. In consequence, the aecial host may be an important factor in the survival and spread of this rust. It must be acknowledged, however, that in the two places where the rust has been observed by us to be most severe, Arlington Experiment Farm, Rosslyn, Va., and Blacksburg, Va., Star-of-Bethlehem is not known as a weed.

SUMMARY

1. The leaf rust of rye, *Puccinia dispersa*, is able to produce aecia on species of Anchusa in the United States.

2. *Anchusa officinalis*, and *A. capensis* are susceptible. *Nonnea rosea* may be infected occasionally with production of pycnia only. The other boraginaceous species tested remained uninfected.

3. *Anchusa capensis* has been found naturally infected by the leaf rust of rye and may become of some importance in the spread of the disease.

4. Apparently unlike the usual situation in Europe, the teliospores of the leaf rust of rye are capable of overwintering, and may germinate the following spring.

5. The leaf rust of barley, *Puccinia anomala*, from four widely separated localities in the United States has been used in inoculation experiments which have resulted in the development of aecia on *Ornithogalum umbellatum*, agreeing with results obtained by Tranzschel in Russia.

6. Although the aecial stage of *Puccinia anomala* has not been found occurring naturally, Star-of-Bethlehem possesses weed-like characteristics in this country which make it a dangerous plant near barley fields.

7. *Puccinia anomala* in its host specialization is distinct from the closely related *Uromyces hordei*. So far as known, neither is able to infect the hosts of the other.

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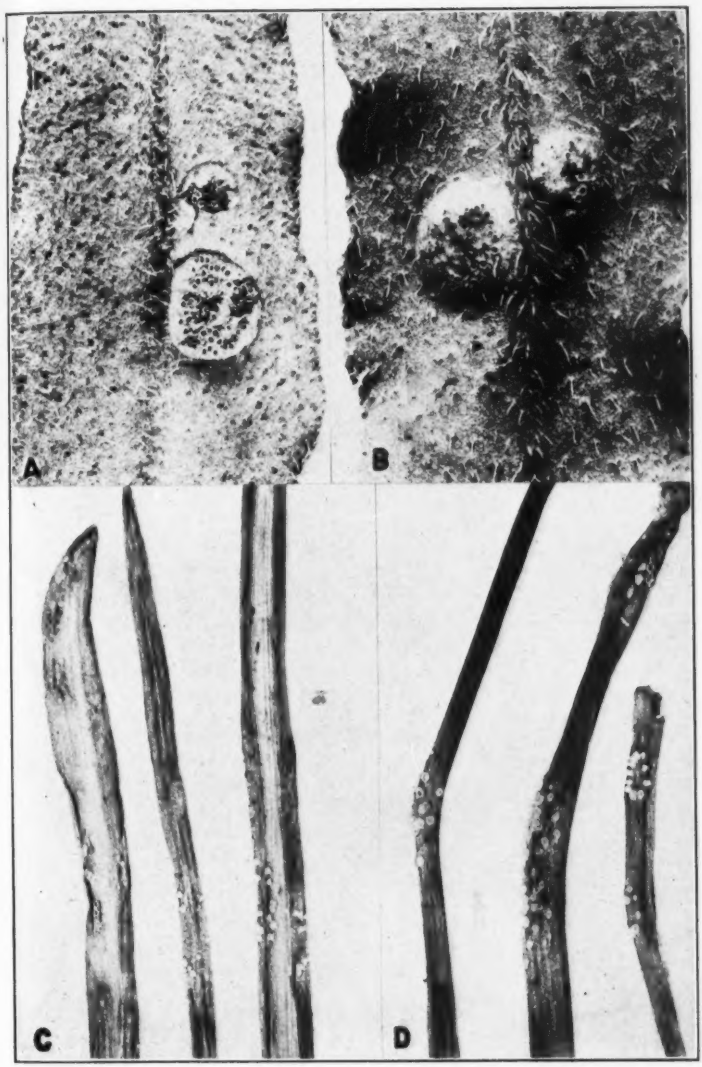
PLATE 1

A.—Aecial stage of *Puccinia dispersa* upon leaf of *Anchusa officinalis*.

B.—Aecial stage of *Puccinia dispersa* upon leaf of *Anchusa capensis*.

C.—Aecial stage of *Puccinia anomala* upon leaves of *Ornithogalum umbellatum*, obtained from infection with teliospores from barley.

D.—Aecial stage of *Uromyces hordei* upon leaves of *Northoscordium bivalve*, obtained from infection with teliospores from *Hordeum pusillum*.


A
B
C
D

FALL SOWING AND DELAYED GERMINATION OF WESTERN WHITE PINE SEED[1]

By W. G. WAHLENBERG

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Experiments to determine the best time to sow seed of western white pine (*Pinus monticola*) have been under way in the northern Rocky Mountain region since 1912, partly in northern Idaho at the Priest River Forest Experiment Station, but mainly at the Savenac nursery on the Lolo National Forest in western Montana. Climate and soil combine to make the problem essentially local, but although the findings may not apply directly elsewhere the observations may be found suggestive.[2]

In order to understand the problem it is necessary to know something about what is termed the "hold-over characteristic" of western white pine seed. Like the other five-needled pines, this species has a strong tendency not to germinate all of its viable seed the first season after spring sowing. At Savenac nursery this delayed or second-season germination is often as high as 50 per cent, and has been anywhere from 4 to 91 per cent of the total number germinating from spring sowings. There are several theories regarding the cause of this habit. Chief among them are, first, the presence of an impermeable seed coat, and, second, the need of an after-ripening process in the embryo.

DISADVANTAGES OF SPRING SOWING AND HOLD-OVER GERMINATION

Table I is based on data secured from spring sowing during three different years and shows the proportion of hold-over germinations to have been high in every case. The figures give no reason to consider early spring sowing any better than late spring for western white pine; nor do seasonal variations in rainfall seem to be directly connected with the results obtained. Precipitation for the year 1914 was average, being but 0.5 inches above the mean amount, while 1915 and 1916 were both fairly moist with 2.27 and 3.59 inches, respectively, above the mean. Artificial sprinkling, of course, is employed to counteract the effect of too little rain in dry seasons.

TABLE I.—*Germination by seasons of spring-sown western white pine*

Time of sowing	Number of plots	Number of seeds	Average percentage of sown seed germinating			Average percentage of total germination which occurred during second season
			First season	Second season	Both seasons	
May 1, 1914	1	4,000	6.0	10.9	16.9	64.5
May 15, 1914	1	4,000	5.1	11.1	16.2	68.5
June 1, 1914	1	4,000	7.3	12.4	19.7	62.9
May 5, 1915	6	5,000	28.8	13.0	41.8	31.1
May 27, 1915	6	6,000	13.4	22.2	35.6	62.3
May 5, 1916	6	6,000	6.0	36.0	42.0	85.7

[1] Received for publication May 21, 1924—issued November, 1924.

[2] The early experimental work in this project was carried on by E. C. Rogers, assisted by P. C. Kitchin. A number of their manuscript reports have been drawn upon to indicate the previous work.

It is very desirable to eliminate this hold-over germination for several reasons. The most obvious objection is that a very irregular stand of seedlings is produced. The seed-bed stand is uneven-aged, uneven in size, and uneven in its resistance to injury. Such irregular stands require heavy culling at the end of the second season or must be kept through the third season with its additional costs for all cultural operations. Either course is undesirable and expensive.

Together with hold-over germination we have to deal with a first-season germination from spring sowings which extends its activity throughout the summer. This aggravates the difficulties just mentioned, particularly the poor resistance. Seedlings originating late in the season are young and tender, and so undergo heavy losses. Death from the damping-off fungi is confined to seedlings three weeks old or less. Young seedlings are scorched by the hot sun of July and August and die more easily from slight drying of the surface soil than do their companions which are a month or two older. This necessitates the heavy expense of shading the seed-beds with lath frames. Then often 20 to 25 per cent of these weak, not yet lignified individuals are lost over winter. Frost heaves the ground in late fall or early spring when the soil is moist and exposed, and the weaker seedlings are heaved out. To avoid this trouble mulching the beds with straw or similar material is necessary, but this in turn results in the breaking of tender stems while applying and removing mulch.

In the endeavor to obviate these difficulties many attempts have been made to hasten the germination of the western white pine seed, among which may be mentioned: Stratification, sowing fresh seed, soaking in hot or cold water or in acids of different concentrations, dry and moist freezing, sowing in hotbeds, sowing of pregerminated seed, and mechanical methods of cracking, piercing, abrading or removing the seed coat. Most of these, however, are dangerously artificial methods which, experience has shown, tend to lower germinative capacity and at the same time do not uniformly accelerate germination.[3]

FALL- VERSUS SPRING-SOWING EXPERIMENTS

Because the results of these trials of spring sowing were all quite unsatisfactory, the possibility of fall sowing was considered. If the hold-over germination could thus be avoided the disadvantage of exposing newly made seed-beds for a longer time to the ravages of bird and rodent enemies, and to frost heaving of seed would be compensated for or could be controlled by other means. In actual practice the losses due to these causes have been small.

During the growing seasons from 1914 to 1916 at the Priest River Forest Experiment Station, observations concerning the best season to sow were taken from plots sown for other studies. In one instance, fall-sown plots had completed 83 per cent of their total germination before the end of May, while less than 14 per cent of the germination in spring-sown plots was complete at that time. Again fall sowings were 17 per cent higher in survival, and produced larger stock in every respect.

In the fall of 1913 and spring of 1914 at Savenac nursery six plots, each containing 2,000 counted seeds, were sown on different dates. Germination from fall-sown plots was complete about 15 days before that from spring-sown plots started. (See fig. 1.)

Twelve plots sown on October 27 and November 4, 1915, and on May 5, 1916, were watched during 1916. Hold-over germinations were much more numerous from spring sowing. Subsequent work has constantly given similar results. (See fig. 2 and 3.)

[3] A full discussion of this subject is given in a manuscript thesis presented in 1917 to the faculty of the Graduate School of Cornell University: ROGERS, E. C. DELAYED GERMINATION AMONG THE FIVE-NEEDLE PINES.

IMPORTANCE OF DATE OF FALL SOWING

The superiority of fall over spring sowing of this species was so marked in the early experiments that it seemed as if the problem were solved, but it soon appeared that the correct time in the fall season for doing the sowing was also very important.

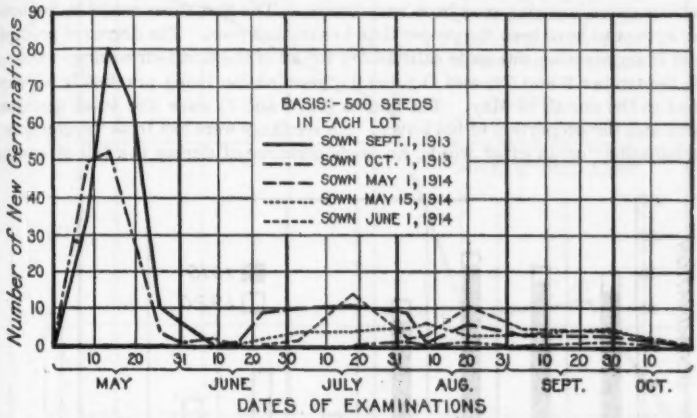


FIG. 1.—Germinative energy of western white-pine seed at Savenac Nursery as influenced by season of sowing

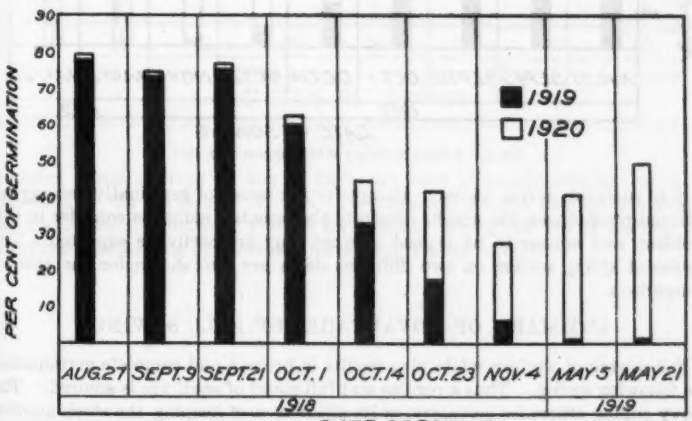


FIG. 2.—Results of sowing of Kanksu seed, 1917

In the fall of 1916, at 10-day intervals from August 21 to October 28, twenty-eight plots of 1,000 seeds each were sown. From the results of this series early fall sowing is indicated as best, in order to reduce to a minimum the germination occurring after the month of June; but a different seed lot, germinating the highest total number, yielded only a small number of germinations after June 30, even from late sowings. Based on the highest number of seedlings germinating promptly the first season, September 1 was the optimum time to sow in 1916.

In the fall of 1917 sowings were made on September 7, 17, 27; October 6, 20, and November 3, with two lots of seed; 24,000 seeds were sown in samples of 1,000. These series showed no startling differences in September sowings, but the October sowings were inferior in promptness of germination and total numbers appearing the first season.

During the fall of 1918, fourteen samples of 1,000 seeds each were sown on various dates, utilizing seed from two sources. The first three weeks in September appear to have been the proper time to sow that year. The degree of promptness of germination was quite satisfactory for all of the first four sowings (August 27, September 9 and 21, and October 1), germination being practically all confined to the month of May. The graphs (fig. 2 and 3) show the total germinations and the proportion of hold-over. No seedlings were lost from "premature" germination; or, in other words, no seeds germinated during the fall of sowing

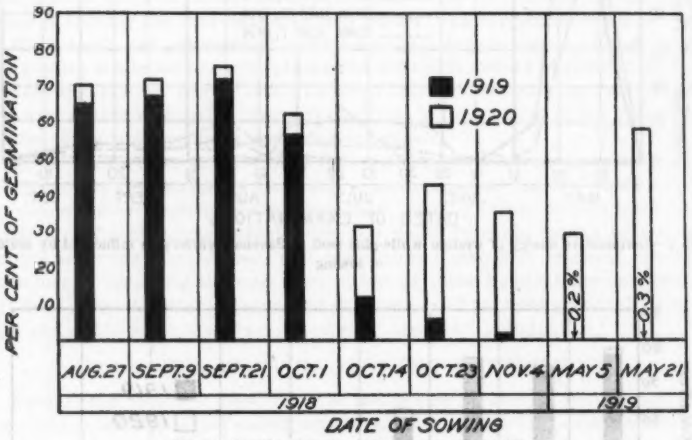


FIG. 3.—Results of sowing of Washington seed, 1917

only to succumb during winter. Except for the factor of germinative energy, or seasonal promptness, the graphs illustrate the essential points to consider in this problem, and appear to be typical of what may ordinarily be expected. The results of spring sowing on two different dates are also shown for the sake of comparison.

SUMMARY OF ADVANTAGES OF FALL SOWING

Fall sowing of western white pine results in prompt and complete germination the following spring. Thus a regular and full stand of seedlings is secured. The heavy culling otherwise necessary, or its alternative of keeping the stock another year, is avoided. No shade or mulch at all is necessary, hence the entire cost of these three operations is saved. Handling the lath shade-frames was formerly a large item, because they had to be removed and replaced several times a season for weeding operations. A cost of $66 for straw and $181.50 for the frames and handling them is now saved each year at the Savenac Nursery as a result of these experiments. In short, it appears that we have avoided completely all of the disadvantages of spring sowings and hold-over germinations.

Figure 4 indicates that the best time in the fall for sowing western white pine seed at the Savenac nursery is during the first two weeks in September. According to the curve, the last few days in August may be as good, but this is not

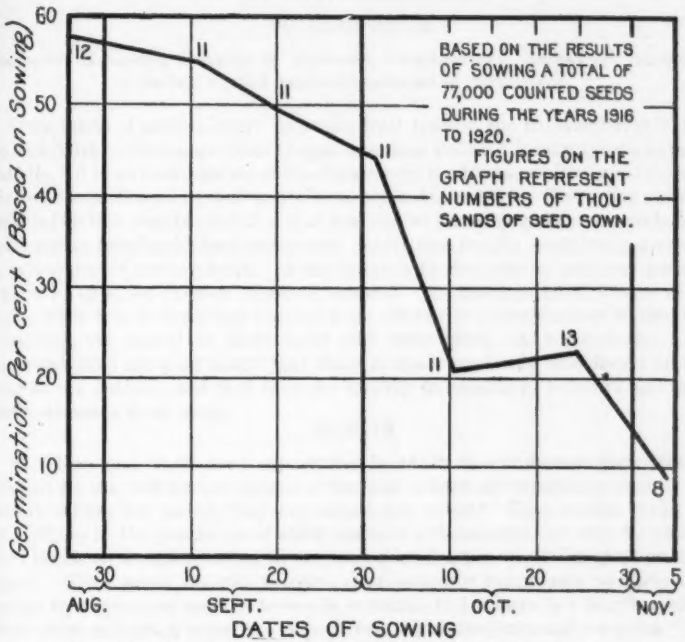
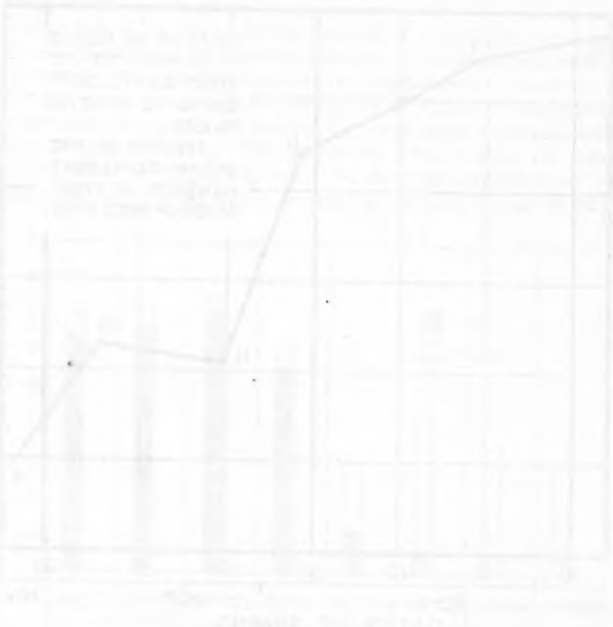


FIG. 4.—Germination of western white pine seed at Savenac nursery as influenced by time of sowing in the fall

certain. Trial sowings in August have not been numerous enough to fix the direction of the curve at that end. Furthermore, August sowings are subject to premature germination; that is, germination during the fall of sowing; and seedlings germinating prematurely do not survive the winter.



THE EUROPEAN HARE (LEPUS EUROPAEUS PALLAS) IN NORTH AMERICA [1]

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Complaints of serious injury to young fruit trees in the Hudson River Valley of New York by European hares (*Lepus europaeus* Pallas),[2] locally known as jack-rabbits, led to an investigation of the situation by the Bureau of Biological Survey, United States Department of Agriculture, beginning in 1916. The writer was first detailed on this work in the fall of that year and at that time gathered considerable information relative to the introduction, habits, distribution, abundance, and economic status of these animals. A second visit to the infested area was made in January, 1918, to develop means of control. In January, 1922, letters of inquiry were sent to State and Federal game officials to collect further information regarding the spread of these hares and their effect on horticulture. The facts gathered leave no doubt that these animals are firmly established on this side of the Atlantic and that they are steadily increasing in numbers and gradually widening their range.

HABITS

The European hares are very similar in habits to our western jack rabbits. Except for the well-known scream of fear and a faint cry sometimes used by the doe in calling her young, they are remarkably silent.[3] They seldom make use of burrows in the ground or of other places of concealment, but rely for protection either on escaping notice by remaining motionless, or, if detected, on their speed. They spend the day in slight depressions, or forms, only partially concealed in a clump of grass or in weeds or brush, but always in a location where their view or hearing is not cut off and from which they can make a quick "getaway." Once started they avoid thickets and make off in the open, depending upon their speed and dodging ability, almost entirely, to escape dogs, although sometimes they are credited with resorting to various tricks to accomplish this.

Their speed exceeds that of an ordinary dog, although some hounds can easily overtake them, and their habit of keeping to the open affords opportunity for observing their rate of travel. Near Lagrange, N. Y., two men reported on numerous tests which they made of this rate of speed whenever they "jumped" hares on the road in front of their automobile. Pressing a hare to its top speed along the road and with an eye on the speedometer they found that the hare traveled at an average rate of 30 to 33 miles per hour. In like manner they found that their collie dog was capable of making close to 30 miles per hour but could never summon the extra burst of speed necessary to catch the hare.

[1] Received for publication Apr. 16, 1924—issued November, 1924.

[2] The European hare resembles closely the black-tailed jack rabbits of the western United States, having the characteristic long ears, much longer than the head, with a black area on the upper surface near the tips, and a conspicuous black area on the upper surface of the tail. It is much larger than our black-tail, an average adult weighing from 8 to 10 pounds or more, and reports of their weighing as much as 15 pounds are on record. The general color of the pelage on the upper-parts is a grizzled, buffy brown; the under-parts are white except on the breast, which is a decided reddish buff.

[3] MILLAIS, J. G. THE MAMMALS OF GREAT BRITAIN AND IRELAND. 3 v., illus. London. 1904-6.

The hare is apparently a solitary animal, more than one seldom being seen at a time, except perhaps in the breeding season. The breeding season, however, is indefinite, as young are said to be found at any time from early in spring till late in fall. There is uncertainty as to the number of litters brought forth each year, but it seems probable that there are at least two, with an average of two to three young to the litter. Barrett-Hamilton states,[4] that the fact that young may be found throughout the year makes it unlikely that there can, as a rule, be less than two or three litters. He further states:

> Most observers have met with small numbers of leverets together up to, at most, four; but larger litters of five, six, seven, or more have occasionally been reported.

He also quotes J. C. Mansel-Pleydell as authority for the statement that, while five were once found together, three is the usual number with vigorous mothers; but with old does only two, and with young mothers one. The period of gestation is probably about 30 days, although definite information on the subject is not available.

HISTORY OF INTRODUCTION

European hares were introduced into America solely as game animals for sport in coursing. They were at first liberated in New York State in a game preserve but were eventually turned loose in the open, as the preserve proved unsuited for coursing, even had the 9-mile fence that was erected been capable of retaining the animals.

There appear to have been five or more successful importations of these hares into America. Probably the most successful, from the standpoint of the subsequent rate of spread and increase, took place about 1893 at Millbrook, Dutchess County, N. Y. In that year a wealthy resident brought in several hundred from Hungary through a New York importing company, under State permit. This importation was the first of a number of his shipments, some of which consisted of 500 animals each, made at intervals of four or five years, the last being in 1910 or 1911. The number of hares introduced was increased by a smaller number imported and liberated on a neighboring estate. The high mortality during transportation made the expense of introduction very great, amounting to about $10 a head.

An earlier importation seems to have been made at Jobstown, N. J., in 1888, but without the impetus of numbers given at Millbrook, N. Y. This importation is reported to have consisted of the brown hare from England (*Lepus europaeus occidentalis* de Winton). An importation is also reported at Bethlehem, Pa., and another at White Plains, N. Y. Information is lacking concerning the source of the hares liberated at these places. An account of the spread of European hares in Ontario, Canada, after being introduced on an estate near Brantford, Ont., has also been received.

DISTRIBUTION AND ABUNDANCE

During the 30 or more years that have elapsed since the European hare first appeared in this country, the increase and spread has been slow but steady. The accompanying map (fig. 1) shows the distribution of the species in the United States as far as reported. The present range might be roughly described as extending from southern Vermont to central New Jersey and eastward 20 or 30 miles into Connecticut and Massachusetts, and to a limited extent westward across the Hudson and Delaware Rivers into extreme eastern Pennsylvania. Reports that are considered reliable have been used in mapping this distribution, but in some cases specimens are not available.

[4] BARRETT-HAMILTON, G. E. H. A HISTORY OF BRITISH MAMMALS. 1: 285-289. London. 1912.

The most notable spread of the hares has been eastward into Massachusetts and Connecticut. One report states that hares have recently been seen as far east as Stratford, Conn. Another report has them in the vicinity of Stafford, in north central Connecticut, but as there is doubt as to the identity of the animals, this record is omitted from the map. In 1915 a hare was shot as far north as Fair Haven, Vt., by the State Fish and Game Commissioner, but as no others have since been seen this far north, the Massachusetts-Vermont line may be considered the northern limit at present (1924).

A very few hares are reported in southern New Jersey, in Salem and Cumberland Counties. Although the report is not sufficiently definite for mapping, it

FIG. 1.—Distribution of the introduced European hare (*Lepus europaeus* Pallas) in the United States.

may be accepted, for, according to reliable information, the hares have been working south from the vicinity of Jobstown in recent years.

Samuel N. Rhoads, in 1903, reported upon the distribution of this hare in New Jersey as follows:[5]

This animal, escaped from domestication, has now become scattered over many localities in the northern half of New Jersey, especially in the pa ts radiating from Columbus. * * * It is now found sparingly in the wild parts of Camden and Burlington Counties, N. J., and is regularly hunted in Bucks County, Pa., during the game season.

[5] RHOADS, S. N. THE MAMMALS OF PENNSYLVANIA AND NEW JERSEY. 266 p., illus. Philadelphia. 1903.

Definite information is lacking as to the extent of infestation in eastern Pennsylvania, although it is certain that hares are scarce there at present. Records are lacking also of occurrences west of the Hudson in New York. A few hares have been reported in the vicinity of Port Jervis, and the animals are reported quite common across the river from Poughkeepsie.

Prof. A. Brooker Klugh, of Queen's University, Canada, writes that the European hares are increasing and spreading rapidly in Ontario and that they occupy some 4,500 square miles adjoining Lake Erie on the north. Information concerning the species is being collected by interested Canadians with a view to publishing a report there on the matter.

The areas of greatest abundance of hares at present are eastern Dutchess County, N. Y., western Litchfield and Fairfield Counties, Conn., and southern Berkshire County, Mass. The hares in New Jersey do not seem to have increased so rapidly as in New York, but they are reported to be numerous in the vicinity of Stelton, Middlesex County.

The abundance of the hares is partially indicated by the number of bounties paid on them by Dutchess County from 1912 to 1917. Bounties paid for the first four years are as follows: For 1912, on 1,987 hares; 1913, on 1,879; 1914, on 3,054; and 1915, on 2,942. The figures for 1916 and 1917 are not available, but slightly over 3,000 bounties in each of these years must have been paid, inasmuch as approximately $4,000 was spent in the six years in paying bounty at 25 cents per head. If the bounty was paid on half of the hares in the county each year, it can be estimated that there are 5,000 hares in Dutchess County, or an average of 6 hares to the square mile. The continued increase of hares, however, in spite of this drain upon their numbers, would probably indicate a much larger population. Based on a study of tracks in the snow near Sheffield, Mass., a population of 20 to 40 hares per square mile has been reported in that vicinity.

DEPREDATIONS

The only serious complaint made against the European hare is the injury it causes to young orchard trees. This occurs only during severe winter weather when the natural food of the hare is not readily obtainable. Under such conditions, however, one hare can do great damage in a young orchard in a short time, and the presence of the animals in a fruit section is a constant menace unless suitable provision is made to protect the young trees.

The first noteworthy depredations of these hares occurred during the winter of 1900, when several young orchards near Millbrook were severely damaged. At this time an unsuccessful attempt was made to collect damages from the person who liberated the animals. The injury by hares was particularly severe during the winters 1909–10, 1912–13, 1915–16, 1917–18, and 1920–21, the unusually deep and persistent snows of these years forcing the animals to depend for subsistence upon the bark of trees and shrubs. The investigation following the winter of 1915–16 showed that a large proportion of the young orchards of Dutchess County had sustained losses from hare depredations. Many orchardists counted trees destroyed by the hundred, a number which often represented entire orchards.

The succulent bark of the younger trees is preferred by the hares, although no tree with smooth bark is immune from their attack. Every tree in one orchard of 200 large 5-year-old apple trees was badly damaged, and it was estimated that the losses in Dutchess County alone during the winter of 1915–16 exceeded $100,000.

The hare shows a decided preference for apple trees. In one large orchard of apple, peach, and cherry trees at Lagrange, N. Y., several hundred apple trees were destroyed, while not one of the peach or cherry trees was touched. Shade trees, ornamental shrubbery, small fruit bushes, and the like are damaged to some extent, but such losses are relatively inconsequential.

The fact that a bounty was placed on the hares indicates that the people of Dutchess County considered them a pest. The county agricultural agent a few years ago voiced this opinion when he stated that hares were the worst pest the orchardists of the county had to contend with, as they were the only one that could not be successfully controlled.

CONTROL MEASURES

The excellence of hares as game, together with freedom from State protection, will do much to lessen the danger of their ever becoming a serious pest, particularly in the more thickly settled orchard sections. Because of the great injury that a few hares can do in a single night, however, it is necessary that orchardists be urged to anticipate an attack and that they be instructed in methods of control. Poisoning and trapping are unsatisfactory, probably because of the difficulty in attracting these wide-ranging animals to a particular bait. In addition to shooting, methods of control include the use of woven-wire fences, individual tree protectors, repellent washes, and winter feeding.

Mechanical protectors often fail at a critical time because of drifting snows; repellent washes deteriorate rapidly; and winter feeding does not always accomplish its purpose. Any one of the three methods, however, will considerably reduce injury in case of attack.

Poultry netting 30 inches high, of 1-inch mesh, encircling the tree, is among the best of the mechanical protectors. Stakes or spreaders should be used to prevent the hares from pressing the wire against the bark and injuring it through the meshes. Various forms of wooden, paper, and cloth protectors are commonly used. They should be removed each spring, however, as they furnish retreats for insect pests when left permanently on the trees.

Of the repellent washes the concentrated commercial lime-sulphur wash is the most promising. Its adhesive qualities may be increased somewhat by the addition of half a pound of cheap glue to a gallon of the lime-sulphur sludge. This is applied to the trunk of the tree with a paint brush. During a severe winter two or possibly three applications may be necessary.

Feeding rabbits in winter to prevent their attack on the trees is practiced on the theory that it is cheaper to feed than to fight them. The most common practice is to leave the winter prunings of the trees scattered about the orchard.

VALUE FOR FOOD AND SPORT

Hares are highly prized as food in Europe and, although in this country opinion is divided as to their palatableness, they find a ready market in the towns and cities throughout their range. Some who are familiar with both European hares and jack rabbits state that the European hare is far superior as food.

A minimum estimate of the present numbers of these hares in this country would be 50,000, which would represent nearly half a million pounds of meat. The meat value of those killed goes far toward offsetting the injury inflicted to orchard trees in the restricted area in which these hares are now found.

European hares are not protected (1924) at any season in New York, Connecticut, or in Berkshire County, Mass.[6] The farmers of Berkshire County made bitter complaint against them to the State Legislature, with the result that all protection was removed in this county. While there is no doubt that hares are a pest in sections where fruit is raised extensively, it is equally certain that they are an important asset as food and game. Their great speed and their characteristic habit of taking to the open when pursued make them a prime favorite in coursing, while they furnish excellent sport to thousands of individual hunters, as was contemplated in their introduction.

[6] LAWYER, G. A., and EARNSHAW, F. L. GAME LAWS FOR THE SEASON 1924-25. U. S. Dept. Agr. Farmers' Bul. 1444, 48 p. 1924. Animal publications.

GROWTH ON CUT-OVER AND VIRGIN WESTERN YELLOW PINE LANDS IN CENTRAL IDAHO[1]

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PURPOSE OF STUDY

The western yellow pine (*Pinus ponderosa*), because of the superiority of its lumber, its abundance, and its accessibility, is the most important commercial timber tree in the Boise, Payette, Weiser, Idaho, and Salmon National Forests in central Idaho. It is of the utmost importance, therefore, that the stands of this species be so managed as to insure an adequate stand of reproduction on cut-over lands and to maintain at its maximum the potential productivity of the type. In order to secure reliable information upon the establishment of a new forest, its growth and development, and to determine the basic principles governing silvicultural practice in this type, the Forest Service established a series of 16 permanent sample plots on the Payette National Forest in central Idaho in 1913 and 1914. The results obtained through the remeasurement of these plots in 1918 and 1919 brought out many points of interest concerning the management of western yellow pine stands in this region which are well worth attention.

GENERAL CONDITIONS ON THE PLOTS

Although these plots were established primarily to secure data on the growth of the reserved trees and natural reproduction in cut-over stands, unfortunately only three of them, plots Nos. 1–3, Table I, had been cut over when the logging operation was suspended in 1914. Nevertheless, they now afford a good comparison between the increment in cut-over and in virgin stands, showing behavior of advance reproduction in the latter. The cutting on these plots approached the method of clear cutting with scattered seed trees, although plot No. 1 had quite a number of trees left because they were too young. The uncut plots Nos. 4 to 16 give a good indication of the increment and mortality which may be expected in virgin stands. Furthermore, they afford interesting comparisons among three important sites common to the Intermountain region.

The sample plots were laid out in representative virgin stands under a variety of topographic and soil conditions.

The soil is a sandy to gravelly loam of granitic origin and is open, porous, and very gravelly on the south aspect. In the basins and on the north aspect the soil is less gravelly and more loamy because of a greater admixture of organic matter.

[1] Received for publication Apr. 29, 1924—issued November, 1924.

TABLE I.—*Number of living and dead trees, 4 inches diameter breast high and over, to the acre*

Plot No.	Aspect	Species	Number of trees at first measurement	Increase or decrease (−) in number of trees	New trees entered	Number of dead trees	Volume of dead trees.[a]	
							Cu. ft.	*Per cent*
1	South	Western yellow pine	28.0	4.2	4.6	0.4	7.7	0.82
2	North	do	6.0	2.4	2.6	.2	11.0	4.66
3	Basin	do	7.8	6.0	6.0	.0	.0	.0
		Average (plots Nos. 1 to 3), cut-over.	13.9	4.2	4.4	.2	6.2	1.16
4	South	Western yellow pine	29.0	1.4	2.0	.6	122.9	2.74
		Douglas fir	3.6	.2	.2	.0	.0	.00
		Total	32.6	1.6	2.2	.6	122.9	2.31
5	do	Western yellow pine	36.6	5.2	6.0	.8	110.6	2.57
		Douglas fir	3.6	.0	.0	.0	.0	.00
		Total	40.2	5.2	6.0	.8	110.6	2.33
6	do	Western yellow pine	21.0	3.2	3.6	.4	94.8	2.86
		Douglas fir	2.6	−.2	.0	.2	10.8	5.76
		Total	23.6	3.0	3.6	.6	105.6	3.01
7	do	Western yellow pine	32.6	1.6	3.0	1.4	198.2	7.93
		Douglas fir	.8	.0	.0	.0	.0	.00
		Total	33.4	1.6	3.0	1.4	198.2	7.31
		Average (plots Nos. 4 to 7), virgin.	32.4	2.8	3.7	.9	134.3	3.30
8	Basin	Western yellow pine	27.4	1.2	1.4	.2	57.0	1.43
		Douglas fir	11.0	−.4	.0	.4	99.8	7.76
		Total	38.4	.8	1.4	.6	156.8	2.98
9	do	Western yellow pine	21.6	5.4	6.0	.6	78.8	2.81
		Douglas fir	19.4	−.4	.4	.8	26.7	1.45
		Total	41.0	5.0	6.4	1.4	105.5	2.27
10	do	Western yellow pine	26.4	−1.8	.2	2.0	64.6	1.90
		Douglas fir	27.0	.4	.4	.0	.0	.00
		Total	53.4	−1.4	.6	2.0	64.6	1.25
11	do	Western yellow pine	16.0	−1.6	.8	2.4	238.9	8.71
		Douglas fir	43.4	.2	.4	.2	7.8	.20
		Total	59.4	−1.4	1.2	2.6	246.7	3.73
		Average (plots Nos. 8 to 11), virgin.	48.0	.8	2.4	1.6	143.4	2.64
12	North	Western yellow pine	16.0	−.4	.2	.6	326.8	12.88
		Douglas fir	38.0	−2.0	.4	2.4	148.4	6.20
		Total	54.0	−2.4	.6	3.0	475.2	9.63
13	do	Western yellow pine	15.8	3.8	4.6	.8	70.4	3.69
		Douglas fir	109.4	4.8	9.2	4.4	36.4	.90
		Total	125.2	8.6	13.8	5.2	106.8	1.80
14	do	Western yellow pine	22.8	−.4	.6	1.0	70.2	2.44
		Douglas fir	30.6	2.8	3.8	1.0	47.3	4.24
		Total	53.4	2.4	4.4	2.0	117.5	2.94
15	do	Western yellow pine	6.2	1.8	1.8	.0	.0	.00
		Douglas fir	57.2	4.2	5.8	1.6	63.3	2.36
		Total	63.4	6.0	7.6	1.6	63.3	1.71
		Average (plots 12 to 15), virgin.	74.0	3.6	6.6	3.0	190.7	4.11
16	do	Western yellow pine	24.2	2.9	3.6	.7	81.5	3.19
		Average (plots Nos. 4 to 16),[b] virgin:						
		Western yellow pine	23.0	1.9	2.8	.9	110.0	3.82
		Douglas fir	21.7	.6	1.3	.7	27.5	2.13
		Total	44.7	2.5	4.1	1.6	137.5	3.30

[a] This includes only those trees which died since the plots were established.
[b] Averages weighted by plot areas for total area of 80 acres.

Like the bulk of virgin western yellow pine forests, the uncut stands are made up mostly of mature or overmature even-aged groups. Some immature groups have, however, become established in the openings in the forest cover caused by the death of old trees. Each of the plots is 5 acres in extent, with the exception of plot No. 16, which covers 20 acres. Plots Nos. 4 to 15 are located on Big Pine Creek, at an altitude of 4,000 to 4,500 feet. Plots Nos. 1 to 3 are on Carpentier Creek, at an elevation of approximately 3,700 feet, while plot No. 16 is located on Poorman Creek, at about the same altitude as plots Nos. 1 to 3. All of these plots are in pure stands of western yellow pine. Of the uncut plots, Nos. 4, 5, 6, and 7 are on ridge tops and south aspects where western yellow pine occurs in practically pure stands. Plots Nos. 8, 9, 10, and 11 are in basins and coves where both species are found in mixture, but with western yellow pine still in the ascendency, while plots Nos. 12, 13, 14, and 15 are located on north aspects, with Douglas fir (*Pseudotsuga taxifolia*) predominating, but with an admixture of yellow pine.

The conditions in the cut and uncut plots as to vigor, mortality, and growth during the five-year period will be brought out in summarizing the results of remeasurement.

INCREASE IN NUMBER OF TREES

The stocking on the cut-over plots is conspicuously incomplete, while the characteristic occurrence of western yellow pine in groups gives an appearance of still greater inadequacy. There are indeed many small saplings on the ground, established before the cutting took place, but these have not yet had time to grow into the 4-inch class, and so did not appear in the records used in this study. The cut-over plots show a somewhat greater increase in number of trees than the virgin plots; an average increase of 4.2 trees an acre during the five-year period as compared with an average increase of 2.5 trees on the uncut plots (see Table I). This difference will in all probability become more pronounced in the course of the next decade or two. It can reasonably be expected that there will be comparatively little loss due to disease and overmaturity in cut-over areas if all defective trees and those of poor vigor be removed at the time of cutting.

INCREASE IN VOLUME

Table II is a summarized statement of the increment on the sample plots during the five-year period. In order to show the actual amount of new wood added each year by growth, the gross increment is also given. Since loss of volume through death of trees can not be avoided under present economic conditions, which render impracticable a return in less than 40 years and possibly longer, it is plain that the figures on net growth, indicating the actual gain or loss in volume, are at present the more significant. It is important to note that without cutting, many of the causes of loss in these overmature stands can not be eliminated.

The table following reveals a wide range in the net increments on the various plots. Other things being equal, the growth on basin plots Nos. 8 to 11 should have been the highest of all, but because of a large number of young thrifty trees (especially Douglas fir) on the northern aspects the growth is actually greater on plots Nos. 13 to 15. The low increment on plot No. 12 is due largely to the high percentage of insect-infested trees. The next lowest rate of net growth per cent is on plot No. 11, where *Dendroctonus* bark-beetle infestation also is serious. Mistletoe, which is prevalent on plot No. 9, does not appear to retard the growth of Douglas fir so much as do bark beetles. Net losses are shown for only three of the uncut plots (plots Nos. 10, 11, 12), though the western yellow pine shows a net loss on five plots in all and Douglas fir on two.

TABLE II.—*Increment per acre of trees 4 inches diameter breast high and over*

Plot No.	Aspect	Species	Volume per acre at first measurement[a]		Periodic annual increment per acre[b]			Percentage of periodic annual cubic foot increment	
					Gross	Net		Gross	Net
			Cu. ft.	*Board ft.*	*Cu. ft.*	*Cu. ft.*	*Bd. ft.*		
1	South	Western yellow pine	942.0	4,780	46.8	45.3	285	5.0	4.8
2	North	do	235.9	1,228	15.0	12.8	75	6.4	5.4
3	Basin	do	424.6	2,352	26.6	26.6	164	6.3	6.3
		Average, plots 1 to 3, cut over.	534.2	2,787	29.5	28.2	175	5.5	5.3
4	South	Western yellow pine	4,483.1	28,584	32.1	7.5	54	.7	.2
		Douglas fir	839.6	4,410	10.7	10.7	63	1.3	1.3
		Total	5,322.7	32,994	42.8	18.2	117	.8	.3
5	do	Western yellow pine	4,306.8	27,212	46.2	24.1	161	1.1	.6
		Douglas fir	437.0	1,902	5.7	5.7	31	1.3	1.3
		Total	4,743.8	29,114	51.9	29.8	192	1.1	.6
6	do	Western yellow pine	3,316.1	21,752	27.0	8.0	37	.8	.2
		Douglas fir	187.5	782	3.8	1.6	9	2.0	.9
		Total	3,503.6	22,534	30.8	9.6	46	.9	.3
7	do	Western yellow pine	2,499.3	14,992	38.2	−1.4	0	1.5	−.1
		Douglas fir	212.8	1,010	2.5	2.5	12	1.2	1.2
		Total	2,712.1	16,002	40.7	1.1	12	1.5	.04
		Average, plots 4 to 7, virgin stands, south aspect.	4,070.6	25,161	41.6	14.7	92	1.0	.4
8	Basin	Western yellow pine	3,978.6	25,454	25.2	10.9	82	.6	.3
		Douglas fir	1,285.6	5,626	18.8	−6.1	−25	1.5	−.5
		Total	5,264.2	31,080	44.0	4.8	57	.8	.1
9	do	Western yellow pine	2,805.5	18,032	17.9	−1.8	−25	.6	−.1
		Douglas fir	1,838.1	8,040	26.7	20.0	96	1.5	1.1
		Total	4,643.6	26,072	44.6	18.2	71	1.0	.4
10	do	Western yellow pine	3,400.7	21,830	19.3	6.4	68	.6	.2
		Douglas fir	1,757.0	7,008	34.4	34.4	164	2.0	2.0
		Total	5,157.7	28,838	53.7	40.8	232	1.0	.8
11	do	Western yellow pine	2,741.4	18,044	9.0	−50.7	−308	.3	−1.8
		Douglas fir	3,880.1	17,034	43.2	41.2	180	1.1	1.1
		Total	6,621.5	35,078	52.2	−9.5	−128	.8	−.1
		Average, plots 8 to 11, virgin stands, basin.	5,421.8	30,267	48.6	13.6	58	.9	.3
12	North	Western yellow pine	2,537.4	16,640	15.5	−66.2	−476	.6	−2.6
		Douglas fir	2,395.0	9,540	17.9	−19.2	−70	.7	−.8
		Total	4,932.4	26,180	33.4	−85.4	−546	.7	−1.7
13	do	Western yellow pine	1,909.4	12,298	12.3	−1.8	−6	.6	−.1
		Douglas fir	4,033.3	15,560	74.9	67.6	270	1.9	1.7
		Total	5,942.7	27,858	87.2	65.8	264	1.5	1.1
14	do	Western yellow pine	2,877.4	19,754	43.7	29.7	170	1.5	1.0
		Douglas fir	1,114.6	4,070	23.0	13.5	52	2.1	1.2
		Total	3,992.0	23,824	66.7	43.2	222	1.7	1.1
15	do	Western yellow pine	1,017.3	6,578	14.4	14.4	102	1.4	1.4
		Douglas fir	2,684.2	10,350	52.0	39.3	156	1.9	1.5
		Total	3,701.5	16,928	66.4	53.7	258	1.8	1.5
		Average, plots 12 to 15, virgin stands, north aspect.	4,642.2	23,698	63.4	19.3	50	1.4	.4
16	do	Western yellow pine	2,556.2	15,964	68.3	52.0	355	2.7	2.0
		Average, plots 4 to 16, virgin stands:[c]							
		Western yellow pine	2,881.1	18,439	35.9	11.7	80	1.2	.4
		Douglas fir	1,291.6	5,333	19.6	13.2	59	1.5	1.0
		Total	4,172.7	23,772	55.5	24.9	139	1.3	.6

[a] Cubic volume includes the solid contents of the entire stem, including the stump, of all trees 4 inches and over at breast height. Board foot volume includes all trees 12 inches and over at breast height, volumes read from a Scribner decimal C volume table for the Payette Forest.

[b] Plots Nos. 1 to 7, inclusive, Nos. 10, and 13 to 16, inclusive, show increment for a 5-year period, while plots Nos. 8, 9, 11, and 12 show it for a 4-year period.

[c] Averages are weighted by plot areas for total area of 80 acres.

A striking difference exists between the rate of growth in the cut-over and the uncut stands. The cubic volume of the cut-over stands has increased at the rate of 5.3 per cent per annum since the plots were established, while the virgin stands show an annual increase of only 0.6 per cent for the same period. With an indicated annual increment of 24.9 cubic feet per acre it is apparent that the loss—by windfall, bark beetles, mistletoe, or otherwise—of one large tree containing 249 cubic feet would completely offset 10 years' growth on 1 acre of virgin timber.

The actual average loss was found to be 6.2 cubic feet an acre for the five-year period in the cut-over stands and 137.5 cubic feet an acre in the virgin stands during the same time. This comparison is gratifying, although the situation still demands improvement of silvicultural practice. It is evident that through a careful selection of trees to be left standing on cut-over areas the maintenance of a desirable rate of volume growth will be made possible.

The percentage of trees showing lack of vigor, as emphasized by Table III, is a good criterion of the general condition of the stands. In this study slight injuries were not considered; the tree had to be decidedly lacking in vigor before being so classified. For example, Douglas fir which showed light mistletoe infection or western yellow pine with slight porcupine injury, if otherwise thrifty, were still classified as vigorous. Since a general lack of vigor and thrift usually precedes death, this will be treated along with mortality.

TABLE III.—*Defective trees on sample plots in 1918 and 1919, with summary of important causes of lack of vigor, in percentages of total living trees and cubic volume at beginning of period*

Cause of lack of vigor	Cut-over plots Nos. 1 to 3		Virgin plots Nos. 4 to 16					
	Western yellow pine		Western yellow pine		Douglas fir		Total	
	Trees	Cubic volume	Trees	Cubic volume	Trees	Cubic volume	Trees	Cubic volume
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Mistletoe infection	2.94	0.57	1.65	0.11	3.37	4.66	1.59	1.47
Suppression					2.35	.16	2.01	.13
Porcupine injury			.65	.14			.34	.10
Defective tops a	.37	.01	2.81	3.06	.90	1.24	1.91	3.07
Unclassified			1.70	1.10	1.80	2.37	1.75	1.44
Total, all causes	3.31	.58	6.81	5.31	8.42	8.43	7.60	6.21

a This group includes such conditions as tops broken in logging, spike tops, and stag-headed trees.

CAUSES OF LOSS

Table IV is a record of mortality from different causes. Of the agents listed as responsible for the death of the trees, the most important are bark beetles, windfall, and mistletoe. In many cases large mature and overmature trees were killed, resulting in a large volume loss.

TABLE IV.—*Causes of death of trees 4 inches diameter breast high, and over, in percentages of total living trees and cubic volume at the beginning of period*[a]

Cause of death	Cut-over plots Nos. 1 to 3		Virgin plots Nos. 4 to 16					
	Western yellow pine		Western yellow pine		Douglas fir		Total	
	Trees	Cubic volume	Trees	Cubic volume	Trees	Cubic volume	Trees	Cubic volume
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Windfall			0.44	0.65	0.75	0.63	0.59	0.65
Insects			2.49	2.85	.46	.97	1.50	2.27
Mistletoe					.29	.27	.14	.08
Suppression			.39	.01	.35	.02	.37	.02
Logging	0.48	0.09						
Porcupines			.11	.001			.05	.002
Unclassified	.96	1.07	.33	.29	1.33	.24	.82	.28
Total	1.44	1.16	3.76	3.811	3.18	2.13	3.48	3.302

[a] This includes only those trees which died since the plots were established.

A maximum loss of 12.87 per cent in the five-year period was found on one uncut plot. Although this loss was occasioned by the death of three large overmature trees, it is nevertheless alarming. Table IV shows that insect attacks, particularly those of the western pine beetle (*Dendroctonus brevicomis*), are responsible for a volume loss over twice as great as that caused by all other agents, and that this has been confined to the virgin stands and to the killing of individuals or small groups of trees. Under normal conditions the infestations are often sporadic, threatening for a year or two, and then decreasing. However, while no serious epidemics have occurred in recent years in this locality, it must not be forgotten that this insect is very destructive to western yellow pine throughout the West and constitutes a real menace which, from time to time, assumes very serious proportions, possibly aggravated by the advent of favorable climatic conditions. Practically all the insect injury noted occurred in trees above the sapling stage, and sufficient insect activity has been shown in virgin stands to warrant foresters giving it careful attention.

On these plots windfall was confined to the mature and overmature trees in the larger diameter classes. On the national forest timber sales this is being partially controlled through the selection of wind-firm individuals for seed trees and through the removal of very tall trees on exposed situations after adjoining trees which protect them are cut. As a matter of fact, wind-throw here was not nearly as serious as it appears to have been in the Whitman National Forest in eastern Oregon (*11*).[1] It is gratifying to note that no wind-throw occurred on the cut-over plots. Since a large proportion of the windfall occurs within four or five years after cutting and the remainder in rapidly decreasing amounts, the exercise of reasonable precaution in marking will undoubtedly eliminate the danger of heavy loss.

Western yellow pine in the vicinity of these sample plots is not infected with mistletoe (*Razoumofskya campylopoda*), although on the basaltic soils of the western division of the Payette National Forest and the nearby Weiser National Forest heavy infections occur. There is a suggestion here of an interesting causal relation between soil type and the local distribution of mistletoe, although the significance and constancy have not been determined. Douglas fir is frequently infected with mistletoe (*Razoumofskya douglasii*). Indeed, on plot No. 8

[1] Reference is made by number (italic) to "Literature cited," p. 1148.

this infection assumed rather serious proportions, causing a loss of 3.09 per cent of the volume. While the loss due to mistletoe is not alarmingly high on any of the plots, the indications are that the death rate will increase from year to year. The information at hand indicates that the cutting of infected trees is the most practical method of control (*6*). Every effort should therefore be made to remove moderately and heavily infected trees when cuttings are made.

Death from suppression obviously results in a relatively small volume loss, although the number of dead trees may be large. The habitual occurrence of western yellow pine in fairly open stands which would seem to predicate a small amount of suppression is, to some extent, counterbalanced by the tendency to form scattered groups, with resultant heavy losses in the smaller diameter classes. The later records on these plots will supply valuable information as to the relative light requirements of the two species, their resistance to overtopping and, on the cut-over plots, their ability to recover upon being released.

It is noteworthy that on the cut-over plots all of the general lack of vigor and thrift is either the result of suppression which took place before the cutting and from which the western yellow pine had not recovered, or is due to the loss of a considerable part of the crown through careless felling of adjacent trees during logging. It would appear that for trees showing a lack of vigor on plots Nos. 1 to 3 inclusive, 3.31 is too high a percentage for recent cuttings, even though the trees comprise only 0.58 per cent of the total cubic volume on the cut-over area. (See Table III.) The need for greater care in logging to reduce the injury to reserved trees and to utilize all suppressed trees which are merchantable can scarcely be overemphasized. The plots show a significantly high percentage of trees in the mature and overmature virgin stands lacking in vigor and consisting of injured, defective, and decadent trees.

Porcupines have caused some injury in certain localities. The actual volume loss due to this agent is small. Saplings are frequently killed by girdling, but large trees are rarely killed. However, the leaders are often girdled, which sets the tree back from 5 to 15 feet or even more in height. New leaders are usually developed, but constrictions and pronounced crooks remain as evidence of the attack, which may even appreciably affect the amount and grade of lumber sawed from trees which recover.

NATURAL REPRODUCTION

One of the main objects of silvicultural management is to secure prompt restocking after cutting. In fact, a forest in which restocking does not follow cutting is doomed ultimately to annihilation. In the forests of the Intermountain region, where artificial regeneration is subject to numerous adversities and an almost prohibitive cost (*7*), natural reproduction is of the utmost importance. Natural reproduction in the western yellow pine type, therefore, has been one of the foremost subjects of investigation in central Idaho. The more important results of these studies have already been published and therefore in the following paragraphs only the principal results will be summarized.

The effect of sheep grazing upon coniferous reproduction and the extent to which this damage can be reduced by more careful use of the range was made the subject of an intensive study by Sparhawk (*10*). Detailed information on germination, survival and causes of death of seedlings with particular reference to grazing injury was secured on 151 small sample plots in the Payette National Forest. This study emphasized the general principle that the extensive browsing of coniferous reproduction and the less palatable forage species is the best evidence that the range is being overgrazed. In many instances on cut-over areas in the course of regeneration it may be desirable to reduce materially or even to eliminate grazing, particularly that of sheep.

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Adverse climatic conditions, while still important influential factors, do not so completely dominate the reproduction problem in central Idaho as they do in the Southwest (*9*). Yet it is considered desirable to retain a sufficient number of trees to preserve forest conditions, in addition to insuring an adequate seed supply, since a heavy opening of the stand increases the danger from wind, excessive evaporation, deficient moisture, and frost injury (*3*, *4*, *5*).

As the result of special studies (*5*) failure of reproduction was found to be due more to high mortality the first three years following germination than to a lack of germination. By far the greatest mortality was found in seedlings less than a year old. Very few over 6 inches high die in any one year. A leaf disease, caused by *Phacidium infestans*, has caused a significant loss among seedlings and saplings of the fairly tolerant Douglas fir and promises to continue as a cause of mortality on the plots. Another leaf fungus (*Hypoderma deformans*) was frequently found to be the cause of fairly heavy losses of western yellow pine seedlings and saplings and ranks along with suppression as a cause of mortality in this species on northern aspects and in basins, especially where the cover is rather dense.

The same field studies showed that the most serious causes of death, aside from the two diseases just mentioned, are in the order of their importance: Drought, browsing and trampling by grazing animals, especially sheep, girdling by rodents, winter-killing, including excessively low temperatures, frost injury, and rodents or birds which bite off the newly germinated seedlings. Seedlings and saplings 15 to 20 years old were occasionally found girdled by rodents. Balanced against this is the fact that rodents render considerable aid in disseminating and burying the seed, thereby promoting favorable conditions for satisfactory germination.

One of the most noteworthy results of these studies has been to emphasize the great importance of advance reproduction. The establishment of advance growth is a long slow process, doubtless requiring as much as 20 years or even more to secure adequate restocking on the poorer sites. Moderately heavy cuttings appear to be fully justified provided advance growth is reasonably abundant and provided healthy, vigorous seed trees are left as insurance against loss of the advance reproduction by fire. It is also evident that efforts to deliberately change the composition of the stand by the method of cutting will produce few tangible results. Western yellow pine is holding its own on potential yellow pine sites and it can be succeeded by Douglas fir only in the tension zone between the two types or in the Douglas fir type. A large number of Douglas fir seedlings can be found, but owing to a heavier mortality extending over a longer period of years, fewer seedlings actually become established than in the case of western yellow pine. Where the two species occur in mixture, as in the basins, the pine in the juvenile stage grows faster than Douglas fir. However, these different rates of juvenile growth do not continue beyond about 40 years of age when Douglas fir surpasses the pine in diameter growth at least.

APPLICATION OF RESULTS IN SILVICULTURAL PRACTICE

When the national forests of the West were placed under Government administration, little exact information on the characteristics and requirements of the important native forest trees was available upon which to base rules for silvicultural practice. As a result of repeated fires, grazing, and insect infestations, the forests were generally understocked and often had a preponderance of overmature and decadent timber and a deficiency of trees of the intermediate age classes from which to select vigorous, thrifty seed trees. All too often advance reproduction was poorly distributed or lacking. In addition there was no sale for defective timber.

Economic conditions have improved to some extent, but still only one cut will be possible for a long time. The marking rules must frequently be based on a compromise between economic necessity in logging practice and the best silvicultural practice.

The distribution of the reserved trees over the area is a matter of great importance. Except for the danger of wind-throw on exposed ridges, it is inadvisable to leave groups of trees of any considerable area untouched, since such blocks are inimical to both acceleration of growth and even distribution of new reproduction. Leaving trees in groups neutralizes the advantageous effects of cutting, and on the better sites may interfere with western yellow pine reproduction. Great care should be exercised in selecting trees for increased volume growth and enhanced quality of the subsequent cut. These conclusions have been substantiated by Dunning's studies (*1*, *2*) in California. He has also clearly pointed out that the crown furnishes the most reliable criterion of what may be expected from reserved trees. A dense, bright-green, pointed crown is indicative of a thrifty tree. Trees with long narrow crowns are generally growing more rapidly than average trees of the same size. As a general principle, it is undesirable to leave mature or overmature seed trees because even on the best sites the death of one large tree will greatly reduce the net increment and the loss will be material of the highest quality. Furthermore, satisfactory growth can not be expected from trees of either western yellow pine or Douglas fir over 30 inches in diameter, even on the best sites in this region.

The marking practice in effect in the central Idaho forests is, in many respects, quite similar to that outlined by Munger (*8*) under the selection system or a modified form of it for the management of the western yellow pine type in eastern Oregon. The present marking practice in central Idaho provides for reserving a considerable nucleus of vigorous immature standards and a sufficient number of sound, thrifty mature trees to make up the necessary quota. These may be desired for the purpose of maintaining the continuity of the forest cover, for increased volume and value increment in a second cut, or to insure adequate seed for regenerating the stand. All defective, diseased, and suppressed trees should be marked for cutting unless needed as fire insurance or seed trees. No defective or diseased tree should be left standing if it is evident that it will not live until the next cutting, unless it is absolutely needed for silvicultural purposes. When it becomes necessary to reserve trees among the larger diameter classes, vigorous, thrifty trees of good form and development should be selected. It is thus evident that the marking on each individual area must be varied to meet the silvicultural requirements of the forest. The importance of careful, intelligent marking of timber on cutting areas can not be overemphasized, since this is the means by which rational silvicultural management is actually secured in practice.

SUMMARY

This report presents the first important results of a growth study which is still incomplete. Permanent sample plots are used to compare cut-over and virgin forests as to condition of stands, mortality, and increment. The cut-over areas are in a much thriftier and more vigorous condition. In the virgin stands the loss of vigor is caused principally by bark-beetle infestations, mistletoe infection of Douglas fir, wind-throw, and suppression. These causes of loss were largely eliminated from the cut-over stands. Porcupines are responsible for a relatively small amount of injury.

The average rate of net volume growth in relation to the volume of the stand is strikingly greater in the cut-over than in the virgin stands. In the latter, the annual loss through death and decay practically nullifies the annual growth.

Great care should be taken in silvicultural markings to reserve, as seed trees, only thrifty individuals which can reasonably be expected to survive until the next cut is made and to continue to grow at a profitable rate. Every precaution should be taken to avoid injury to advance growth at the time of logging because of its supreme importance in regenerating the uneven-aged forests of western yellow pine promptly.

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PLATE 1

A.—Cut-over stand of western yellow pine on a south-facing slope. The presence of saplings and small-sized trees gives the appearance of a "selection" cutting. The opening and the absence of a ground cover of shrubs is typical of ridges. Payette National Forest (plot 1).

B.—Heavy cutting on a north-facing slope. After six years reproduction is still deficient on this area (plot 2), a condition evidently due to the inability of the small-sized trees left after cutting to supply sufficient seed.

A

B

A

B

PLATE 2

A.—The advance reproduction is deficient in this virgin stand of western yellow pine on a south-facing slope of moderate steepness. Ground cover is not dense on such sites and the lack of advance reproduction emphasizes the need for care in marking such stands, if prompt restocking is to be assured. Payette National Forest (plot 5).

B.—A steep south slope necessitates light cutting as in the case of this mature stand of western yellow pine on a steep south slope (plot 6). On such situations, particularly near the tops of the ridges, cutting should be light to avoid heavy wind-throw and excessive drying of the soil. Payette National Forest.

PLATE 3

A.—Large, thrifty trees must be reserved to reseed such areas. A mature stand of western yellow pine and Douglas fir typical of the basins in the Payette National Forest. Advance reproduction is deficient on this area (plot 8), except in the openings. When such stands are cut over, satisfactory restocking must depend upon reseeding by vigorous trees of large diameter left for that purpose.

B.—Abundant advance reproduction under a mature stand. Unusual care should be exercised in cutting such stands as this one of western yellow pine and Douglas fir to release and preserve the abundant young growth, particularly of yellow pine. Payette National Forest.

A

B

SOME FACTORS AFFECTING REPRODUCTION AFTER LOGGING IN NORTHERN IDAHO[1]

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In the virgin forest of northern Idaho, natural reproduction of desirable forest tree species after cutting is often very scant and disappointing. Not only do the defective and prolific weed species of western hemlock and grand fir prevent good establishment of western white pine, but also good reproduction and establishment of the pine on exposed slopes and flats is in most cases very difficult to obtain. The latter difficulty comes about either through failure of the seed to germinate or because the seedlings, many of which may spring from seed already in the forest floor at the time of cutting, do not withstand the hot, dry summer weather and the drying out of the soil.

In order to throw light on this question of germination and survival, certain investigations, recorded in this paper, were made by the Priest River Forest Experiment Station in northern Idaho. These have to do with the effect of large clearings as compared with shelterwood and the uncut forest, germination of seed and establishment of seedlings on the various surfaces found after logging, and the influence of different aspects.

INFLUENCE OF LARGE CLEARINGS

In the first place, the mere opening of the forest by cutting affects the physical conditions of the site in a very marked degree, both by increasing air and soil temperature, evaporation, and rate of transpiration, and by causing extremes or dangerous fluctuations which prove injurious to seedlings. In order to gain information on this point, studies were conducted during the dry and warm summer of 1919 on sites offering comparisons of three different conditions: (1) 250 feet within an old and uncut virgin forest of western white pine (*Pinus monticola*), western red cedar (*Thuja plicata*), western larch (*Larix occidentalis*), and grand fir (*Abies grandis*); (2) at a point 400 feet east of the first site under about one-third overhead cover provided by leaving defective hemlock (*Tsuga heterophylla*) and other species; and (3) on a wide and complete clearing to the south of the other two sites where all of the overhead trees were removed and the site fully exposed to sun and wind. These three locations were all found on a broad river bench at an elevation of 2,300 feet in the Priest River valley.

Each station was equipped with maximum and minimum air thermometers of the United States Weather Bureau pattern, and air thermographs were placed in the stations in the open and in the uncut forest. These instruments were placed 4½ feet above the ground within suitable shelter boxes. At each station there was one psychrometer and one Livingston cylindrical porous-cup atmometer. The latter was placed 6 inches above the ground, the bottle containing the water being entirely below the surface.

Daily soil temperature readings and semiweekly soil-moisture samples were obtained at a depth of 6 inches near each station on a bare plot of ground 1

[1] Received for publication June 19, 1924—issued Nov., 1924.

meter square, away from all roots. The records, except those of soil moisture, were taken at 5 p. m. each day from July 12 to August 31. A summary of the data obtained is given in Tables I and II.

TABLE I.—*Physical conditions within the forest and on large clearings*

(Summaries of measurements July 12 to August 31, 1919)

Stand conditions	Air temperature		Relative humidity 5 p. m.	Daily evaporation	Soil temperature at depth of 6 inches	Soil moisture at depth of 6 inches
	Average maximum	Average minimum				
	°F.	°F.	Per cent	Cc.	°F.	Per cent
Open	87.7	39.5	30.7	35.3	63.2	[a] 31.6
						[b] 36.7
One-third cover	85.3	44.3	33.0	26.9	59.9	[a] 23.5
						[b] 28.1
Uncut	80.3	46.2	35.0	16.0	54.7	[a] 28.0
						[b] 32.6

[a] July measurements. [b] August measurements.

TABLE II.—*Daily march of air temperature in the forest and on large clearings*

(Thermograph records for August in degrees Fahrenheit)

Location	A. M.						P. M.					
	2	4	6	8	10	12	2	4	6	8	10	12
In open	42.7	40.4	39.5	50.4	64.5	76.7	82.4	83.3	78.5	61.2	51.0	46.1
In forest	50.7	47.9	46.0	47.6	57.7	67.4	74.1	76.1	73.6	65.9	56.6	53.9

The records in Table I show that the high points of air and soil temperatures in the open average from 7 to 8 degrees higher than within the uncut forest, and that the range in air temperature in the open is 48.2 degrees but only 34.1 degrees in the forest. These differences, accentuated by a lower humidity and greater evaporation, which naturally intensify the effect of drought on exposed surfaces, are factors which must be carefully weighed in providing suitable environments for seedling establishment of western white pine or other moisture-loving species. It should be stated that the slightly lesser soil moisture observed under the forest than on the open plot is considered due to root activity under the forest, for every part of the soil under the mature forest comes within reach of certain roots, whereas on the open plot where no trees exist, root competition is eliminated. In neither case, forest or open, did soil moisture conditions show any material difference or appear critical. The injurious effects of the exposure are expressed rather by greater evaporation and presumably much higher rates of transpiration, higher soil temperatures, or greater weed and grass competition on the open area than elsewhere.

There is other evidence that complete openings one-quarter mile or more in extent render the site very unfavorable to natural establishment of hemlock and cedar and Engelmann spruce (*Picea engelmanni*), and somewhat precarious for white pine seedlings. This is expressed by the disappearance in such locations of many of the typical moisture-loving plants which usually grow underneath and among the white pine stands, such as *Coptis trifolia, Cornus canadensis, Aralia nudicaulis, Asarum caudatum, Circaea pacifica, Claytonia perfoliata,* and *Galium boreale;* and the appearance of species less dependent upon soil

moisture and shelter such as *Rubus strigosus, Lonicera utahensis, Linnaea borealis americana, Spiraea lucida, Ceanothus velutinus, Salix scouleriana* and a great many grasses and sedges frequently found in the more open western yellow pine (*Pinus ponderosa*) and Douglas fir (*Pseudotsuga taxifolia*) stands.

This change in the physical condition of the site, brought about by clear cutting of the forest, has been observed by the writer in numerous instances over a period of years. Such clearings ordinarily show poorer natural restocking of western white pine and other moisture-loving species compared with north and east aspects; also relatively fewer seedlings in the presence of heavy sod and dense weeds than under partial shade or among lighter vegetation. On one such recently clear-cut flat the seedlings which germinated from seed in the duff were counted for three consecutive years. These showed a mortality of about 50 per cent, although they had no weed or grass competition. Numerous counts and observations, made on large clear-cut areas from eight to twelve years following cutting, show natural reproduction at a standstill except where sheltered by the border of the uncut timber. What takes the greatest toll of young seedlings and creates the most critical physical barrier to natural reproduction on large clearings and south and west aspects in northern Idaho is the fact that the prevailing and desiccating westerly winds in summer always strike the warm and sunny places. Naturally this hindrance, by exposure, drought, competition, and dense vegetation, becomes more acute and more dangerous to natural regeneration on sites with poor soil and on southerly exposures than on sites with deep, moist soil or on northerly aspects or in small sheltered spots such as result from partial cutting of the forest. The influence, therefore, of large clearings on flats and sunny slopes is often to defeat altogether the reestablishment of western white pine and other moisture-loving species.

INFLUENCE OF SURFACES ON GERMINATION AND SURVIVAL

To test out the influences of the different kinds of surfaces such as ordinarily are found on a logged area, seven seed beds, each 4 feet square, were sown with western white pine seed in the fall of 1918. The tests embraced surfaces of ashes, fresh soil, charcoal and ashes, wood and duff charcoal, partly burned, and unburned surfaces. Each bed contained 2,000 fresh white pine seeds and was protected from birds and rodents by proper screens. The ash surfaces were the result of brush burning and the bare soil surface was prepared by raking off the needles, duff, and humus layer. In all cases the seed was sown without raking it in. The results are given in Table III.

TABLE III.—*Germination and survival of western white pine on different surfaces*
(Sowing of 2,000 seed per plot in the fall of 1918)

Date of germination	Germination percentages by types of surface conditions						
	Unburned deep duff	Partly burned deep duff	Unburned duff and decayed wood	Mixture of charcoal and partly burned duff	Bare loose soil	Charcoal and ashes	Deep loose ashes
1919	0.2	4.9	0.3	0.6	12.7	10.2	17.7
1920	9.5	53.9	12.8	9.9	47.9	28.7	15.2
Total	9.7	58.8	13.1	10.5	60.6	38.9	32.9
Percentage of survival 1921	96.9	100.	53.4	36.5	48.2	62.5	95.8

The best germination took place on partly burned deep leaf duff and bare soil, and the burned surfaces in general showed better germination than the unburned plots. The fairly prompt germination on deep ashes is worth noting. This is the surface that gave greatest germination the first season. On the unburned

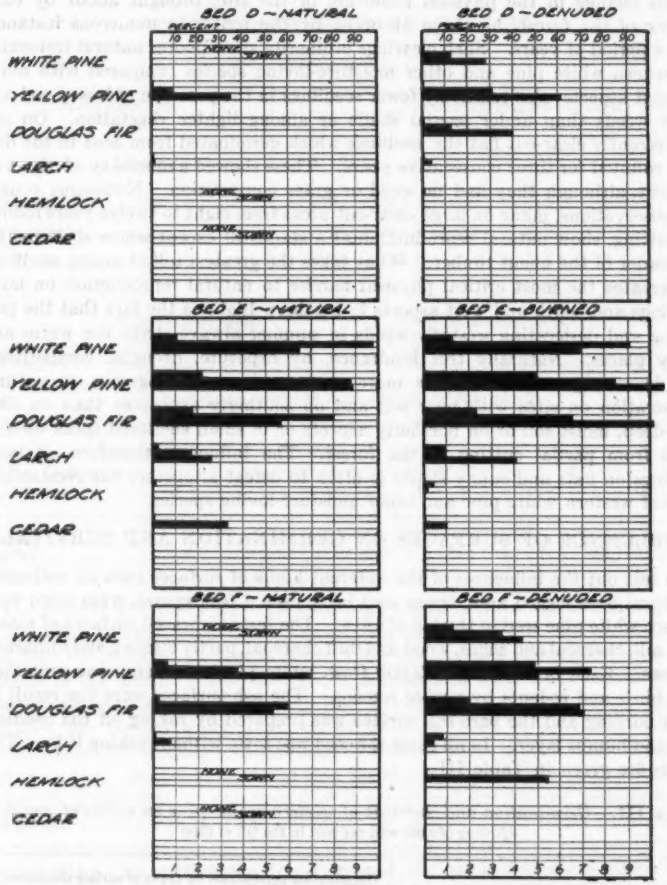

FIG. 1.—Some earlier results of germination and survival on different surfaces, by species. The three horizontal bars opposite each species indicate (1) germination, per cent of seed sown; (2) survival, spring of 1915, per cent of germination; (3) average height in inches, fall of 1917

surfaces the second year's germination, which is a marked characteristic of western white pine, was by far the strongest; in fact, practically all of it occurred during the second season. In the matter of survival the differences are less distinct, but, taking it all in all, the burned surfaces showed results fully as good as the unburned. It should be mentioned also that the seedlings on the ashes and the bare soil were taller and sturdier than those on unburned ground, and that the survival of the trees germinating on the burned surface showed an exceptionally high percentage.

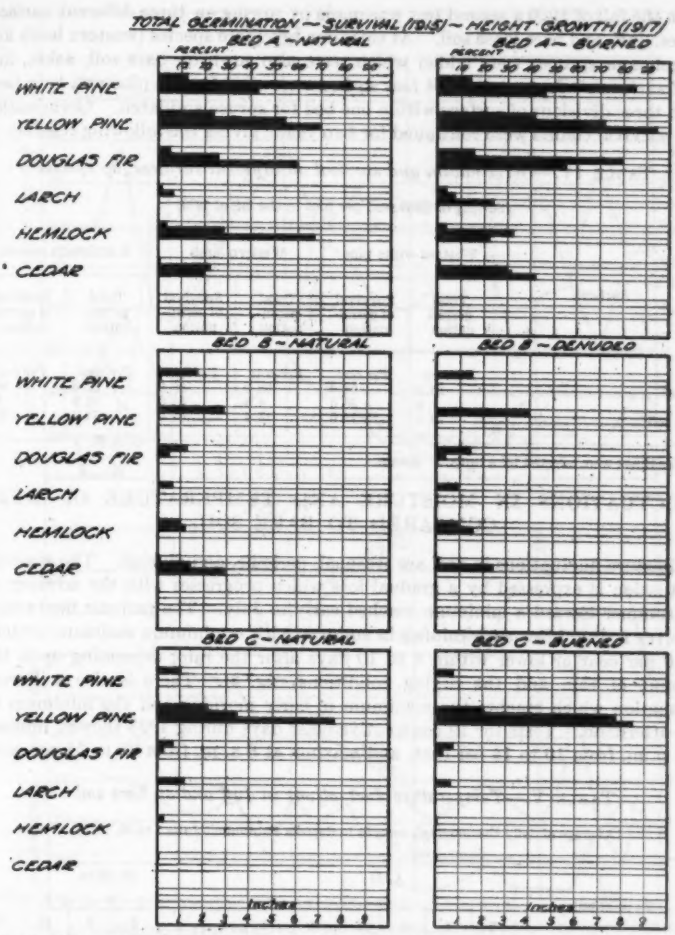

FIG. 2.—Some earlier results of germination and survival on different surfaces, by species. The three horizontal bars opposite each species indicate (1) germination, per cent of seed sown; (2) survival, spring of 1915, per cent of germination; (3) average height in inches, fall of 1917

Some of the earlier results are shown in figures 1 and 2.

Satisfactory germination of these species took place both on the bare, loose mineral soil and on the ashes, and the survival on both was good. The duff surfaces show poor results for all three species and more distinctly so here than in the 1918 series.

Even in the absence of exact measurements of moisture content and surface temperature, one would surmise that the great fluctuations in moisture taking place on duff surfaces would be a disadvantage to seed germination. It is of interest to record a few of the more outstanding and characteristic results of a great many tests of duff moisture contents and fluctuations made at the Priest River Forest Experiment Station by H. T. Gisborne and the author, dating back to 1913.

In the fall of 1920 a second test was made by sowing on three different surfaces ashes, duff, and bare loose soil. At this time two more species (western larch and Engelmann spruce) were added which were also sown on bare soil, ashes, and duff surfaces. Separate beds 4 feet square were used for the pine and long beds with three divisions of surface within one bed for spruce and larch. Germination and survival counts were continued for two years, giving the following results:

TABLE IV.—*Germination and survival on different surfaces, by species*

(Sowing of 2,000 seed per plot in the fall of 1920)

Surfaces	Western white pine		Western larch		Engelmann spruce	
	Total germination	Survival of germination	Total germination	Survival of germination	Total germination	Survival of germination
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Ashes	24.1	94.0	20.0	36.0	24.0	64.0
Duff	1.6	58.0	4.3	35.0	14.0	0.0
Bare soil	24.5	[a] 13.0	13.4	64.0	35.0	67.0

[a] Seedlings were affected by a fungous disease.

FLUCTUATIONS IN MOISTURE AND TEMPERATURE OF DUFF COMPARED TO BARE SOIL

Moisture fluctuations in duff are seasonal, periodic, and diurnal. The seasonal fluctuation is expressed by a gradual loss which progresses with the advance of the summer toward a minimum reached early in July. The periodic fluctuation involves a drop after each rainfall in summer with a minimum moisture content of 10 per cent or lower within 8 to 10 days after the rain, depending upon the amount of rain and the drying conditions (fig. 3). There is also a diurnal fluctuation which reaches the maximum in early morning and the minimum in late afternoon. Tests for 19 consecutive clear days during 1923 showed minima at 5 p. m. from 10 to 14 per cent, and maxima at 8 a. m. from 20 to 24 per cent.

TABLE V.—*Temperature fluctuations in duff and on bare soil*

(Average of daily thermograph records in degrees Fahrenheit June 1 to 30, 1921)

Type of soil	A. M.						P. M.					
	2	4	6	8	10	12	2	4	6	8	10	12
Duff	55.8	54.9	53.5	54.3	56.8	61.2	66.1	65.0	63.3	61.1	58.8	56.8
Bare soil	51.4	50.1	50.4	53.8	60.9	63.1	70.3	65.5	62.1	58.4	55.3	53.2

Naturally, the duff dries out to a greater depth than ash or bare soil surfaces so that seedlings with short and tender roots, like spruce, hemlock and cedar, are often overtaken by drought before the roots become properly established in the underlying soil. In soil and ash surfaces saturation may be as great as in duff but drying out is much less rapid. In this case the entire underlying soil must become dry before surface moisture gives out. Again, moisture adheres closely to the seeds when sowed in ashes or on soil, in that these become imbedded in, or coated with, soil or ashes and are at all times in close contact with the surface. From the standpoint of moisture, therefore, soil and ash surfaces present more favorable seed beds than duff or loose litter.

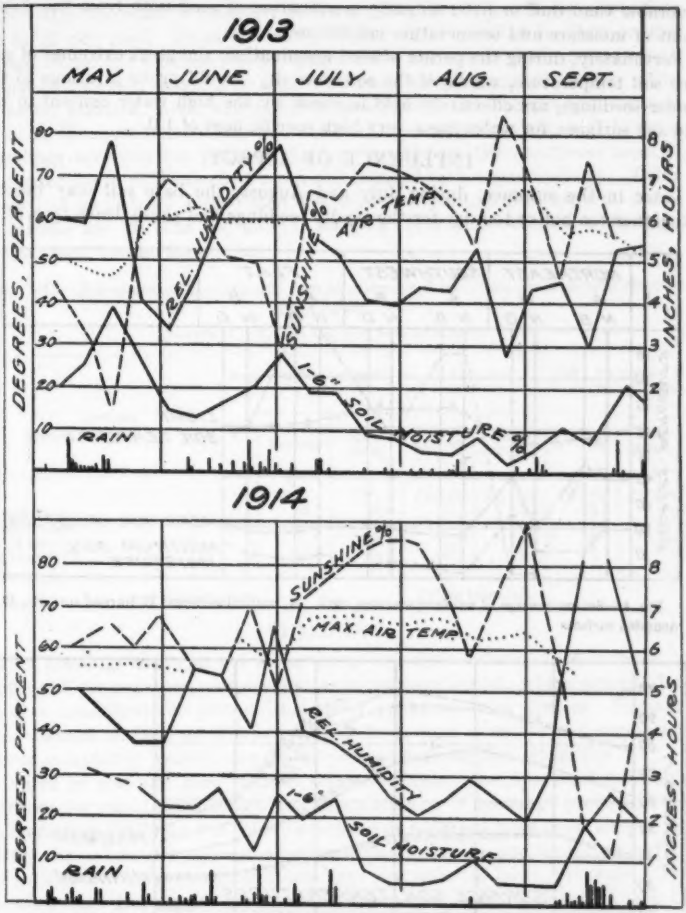

FIG. 3.—Correlation of relative humidity and soil moisture, Priest River Experiment Station

But since moisture alone does not induce germination, the variations in temperature must also be tested. With this in view, soil thermographs were used in the 1920 series, one bulb being placed directly under the surface of the bare soil and another similarly placed under a duff surface. Continuous records were obtained from April 15 to June 30, 1921. This covers the germination period. Results from June 1 to 30, the period of most active seed germination, are given in Table V.

Between 9 a. m. and 3 p. m. from June 1 to 30, the soil surfaces average from 2° to 5° F. higher temperature than the duff. These are averages for clear and cloudy days alike; during sunny days there were differences from 7° to 10°.

This higher temperature on soil surfaces is naturally explained by the lower specific heat of soil, 0.2, as compared with duff, 0.3. The greater heating during the day is a condition which greatly stimulates germination on bare soil and ash surfaces over that of duff and litter. Soil and ash surfaces are, therefore, more

favorable than duff or litter to early germination of seed both from the standpoint of moisture and temperature conditions.

Fortunately, during the period of seed germination, the great extremes of surface soil temperature, which, if the soil were dry, would prove injurious to the tender seedlings, are effectively held in check by the high water content in soil and ash surfaces, for water has a very high specific heat of 1.0.

INFLUENCE OF ASPECT

Later in the summer, during July and August, the bare soil may become altogether too hot or too dry for the tender seedlings and cause death by wilting

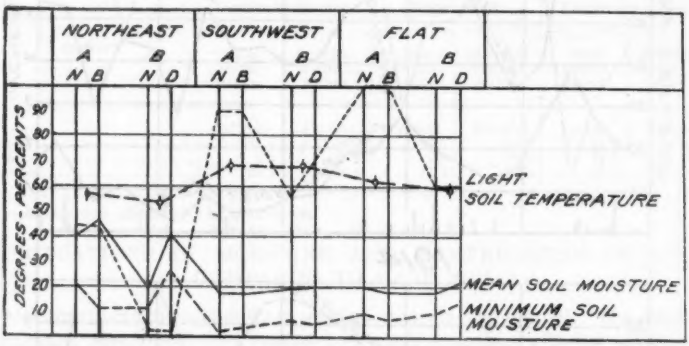

FIG. 4.—Season average of soil temperatures, 1913. N, natural surfaces; B, burned surfaces, D, denuded surfaces

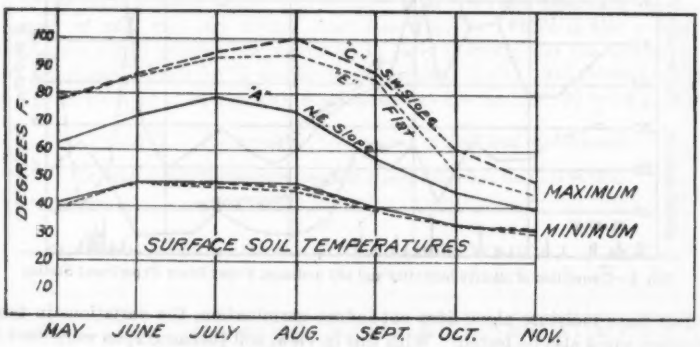

FIG. 5.—Season average, 1913, beds A, C, and E

or burning of the stems, or for want of sufficient soil moisture. It is from this standpoint that aspect and the degree of slope play an important part on seedling survival. The figures in Table VI merely summarize the high points of data obtained over a period of five years, from 1911 to 1916, on a northeast slope, a southwest slope, and a flat, all within one-half section of land. (See also fig. 4.)

From the data in Table VI, and as shown in figure 5, it is seen that the absolute maximum surface temperatures and moisture content on the northerly slope never become critical for seedlings. But on the exposed flats and on southerly aspects the maximum surface temperatures have registered from 115° to 125° F., and the soil moisture has been as low as 3.4 to 0.5 per cent. These extremes ause death both by direct contact and by excessive evaporation, for on such

very hot days the relative humidity of the air reaches the extremely low points of 10 to 20 per cent. From the many sowing tests installed in 1913 on the northeast slope, the southeast slope, and the flat with sandy soil, in the course of which 84 different samples of seed were used, germination of western white pine, cedar, hemlock, larch, and grand fir was proved to be as good on exposed sites as on the most favored aspects; but only western yellow pine and Douglas fir seedlings survived the peak of the hot weather on the southwest aspect.[1] The greater death rate occurred when soil became very warm and dry. Often sites such as result from unrestricted clearings on south and west aspects, therefore, become too dry and too hot for seedling survival of western white pine and other moisture-loving species.

TABLE VI.—*Extremes of surface soil temperature and moisture on different aspects in August*

(Records of 1913, 1915, and 1916, northern Idaho)

Location	Surface temperature		Surface soil moisture		Soil moisture at 6–12 inches depth	
	Average	Maximum	Average	Minimum	Average	Minimum
	°F.	°F.	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Northeast slope	60.2	85.0	43.6	10.7	42.9	11.9
Southwest slope	76.9	125.0	7.7	0.5	14.6	2.6
Larch fir flat [a]	75.3	115.0	7.0	2.1	13.4	5.0
White pine flat [b]	75.9	118.0	19.1	3.4	38.3	11.0

[a] Soil of sandy loam. [b] Clay loam soil.

SUMMARY

Large openings made in the forest cover by clear-cutting cause increase in air and soil temperature, evaporation and moisture deficit, which present unfavorable conditions for reestablishment of moisture-loving species. Furthermore, changed surface conditions resulting from large openings and vegetation on areas completely cleared may defeat natural regeneration altogether.

Surfaces of ash and bare mineral soil when loose offer the most favorable conditions for rapid germination and establishment of seedlings provided seed is immediately available, but where overhead or adjacent trees must supply the seed for natural restocking over a period of years, a loose and protected surface such as is provided by needle duff and light vegetation is more favorable. The tests show that Engelmann spruce and western larch, and in this class may be placed western red cedar and western hemlock, germinate well on duff surfaces, but have very poor survival on account of their short roots. The extremely high surface soil temperatures which occur on cleared and exposed flats and south slopes are injurious to establishment of seedlings of western white pine, cedar, and hemlock, and this explains the general scarcity of these species on sites exposed to sun and wind and the difficulty of restocking these after clear-cutting on a large scale.

A method of cutting which would provide smaller openings and partial shade or shelter would produce better silvicultural results.

[1] While no experimental data are available bearing on the effect of direct heat on unlignified seedlings, Dr. Paul Sorauer (Manual of plant diseases, tr. by F. Dorrance. Ed. 3, v. 1, illus. [Wilkes-Barre, Pa. 1914–1922]) states that a temperature of 104 to 122° F. causes death of plant leaves by burning. Prof. J. W. Toumey and E. J. Neethling (Some effects of cover over coniferous seed beds in southern New England, Yale Univ., School Forestry Bul. 9, 39 p., illus., 1923) record having obtained surface temperatures up to 130° F. which caused death-forming lesions on the unlignified seedling stems. The author has observed similar results with Engelmann spruce in the greenhouse at temperatures between 110° and 120° F.

THE EFFECT OF CYANAMID AND RELATED COMPOUNDS ON THE NUMBER OF MICROORGANISMS IN SOIL [1]

By F. E. Allison

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INTRODUCTION

The decomposition of cyanamid in soil is directly dependent in certain stages of the process upon the numbers, species and efficiency of the soil microorganisms in bringing about the desired changes. The conversion of calcium cyanamide into acid cyanamide, then free cyanamide and the latter into urea is thought to be chiefly a series of chemical changes in which soil colloids play an important rôle. The conversion of urea into ammonia and the oxidation of the latter to nitrites and nitrates are biological phenomena. It is, therefore, important to know the effect of cyanamid and its possible transformation products upon the numbers of soil organisms. It was with this idea in view that the experiments reported below were undertaken.

EXPERIMENTAL

Five groups of experiments were carried out at intervals lasting over a period of about 18 months. The methods used in the first of these were considerably different from the later work and are, therefore, discussed separately.

EFFECT OF CYANAMID AND OTHER NITROGENOUS COMPOUNDS ON BACTERIAL NUMBERS

A Susquehanna loam soil of relatively high fertility was secured from a garden, sieved while moist, and spread out in a thin layer to dry where not exposed to direct sunlight. Sixteen samples of 500 gm. each were than weighed out into 800 cc. beakers. To each was added 5 gm. of precipitated calcium carbonate and the different nitrogenous materials shown in Table I, these materials being uniformly mixed with the soil. The optimum moisture content of the soil was then determined and this quantity, 21.5 cc. of water per 100 gm. of soil, was added regardless of treatment. It was realized that the actual quantity of available moisture would be less in the soil receiving cyanamid, because of its drying properties, but it was decided not to make a correction for this factor. During the incubation period, which began on February 5, 1921, the beakers were kept covered with watch glasses and maintained at room temperature. Water was added at frequent intervals to replace that lost by evaporation.

The first samples for analysis were taken from the center of the beakers at the end of 16 days by means of a sterile cork borer, having a diameter of about three-fourths of an inch. These soil samples were added to flasks containing sterile tap water, then shaken at intervals covering a period of a half hour and agar plates poured according to the usual bacteriological technique.

[1] Received for publication April 28, 1924—issued Nov., 1924.

The medium used was Lipman and Brown's modified synthetic agar, havin the following composition:

	Grams
Dextrose	10. 0
$MgSO_4$	. 2
K_2HPO_4	. 5
Peptone	. 05
Agar	15. 0
$FeSO_4$	Trace.
Distilled H^2O	1, 000 cc.

The quantity of di-potassium phosphate used was just sufficient to make the reaction optimum for bacterial growth and hence no adjustment was necessary.

After an incubation period of seven days at room temperature, the number of colonies appearing on each plate was determined. It happened that the dilution used was somewhat low for the most accurate results, and furthermore, several plates were overrun by quick-growing fungi. The large number of plates poured for each treatment, namely 10 (duplicate beakers of soil and 5 plates from each beaker) largely overcame these sources of error, making the final results as accurate as may ordinarily be expected from quantitative bacteriological work. The average results for the various treatments, expressed in terms of millions of microorganisms per gram of moist soil, are given in Table I.

The second set of samples for biological analysis was taken from the beakers of soil 31 days after the beginning of the incubation period. Plates were poured as previously except that a higher dilution was used. Three plates for each dilution were poured, making a total of 12 for each soil treatment. The average figures are given in Table I, together with those from the first sampling.

From the data as given, it will be observed that there was no constant relationship between bacterial numbers as shown by the methods used and the known effects of the various materials on plant growth. All of the materials used increased the numbers of soil organisms. Of these materials, the two which are known to be the best sources of nitrogen for plants under widely varying conditions, namely, ammonium sulphate and urea, produced only moderate stimulations. In this connection, it is necessary to bear in mind that the urea bacteria do not grow well, if at all, on media such as the one used which did not contain urea. These urea bacteria probably were increased, but this could not be discovered by the method used. Dicyanodiamid, which is unavailable as a fertilizer and injurious to nitrification, produced a moderate increase in numbers. Guanylurea sulphate, another material of doubtful agricultural value, caused greater increases than either of the three materials just mentioned. Cyanamid proved to be remarkably effective in increasing the rate of multiplication of the soil organisms, this increase amounting to 57.8 per cent within 16 days and 320. 2 per cent after 31 days. On the other hand, very large quantities (2 per cent) practically sterilized the soil or at least so weakened the organisms that they were unable to develop on artifical media.

TABLE I.—*Numbers of microorganisms in soil following applications of cyanamid and related compounds*

Treatment	Millions per gram	
	16 days	31 days
No nitrogen	22. 3	22. 8
Ammonium sulphate 0.1 per cent	22. 5	31. 7
Urea (N◇0.1 per cent ammonium sulphate)	30. 9	27. 2
Dicyanodiamid (N◇0.1 per cent ammonium sulphate)	29. 2	25. 6
Guanylurea sulphate (N◇0.1 per cent ammonium sulphate)	28. 6	51. 6
Cyanamid (N◇0.1 per cent ammonium sulphate)	35. 2	95. 8
Cyanamid (N◇0.5 per cent ammonium sulphate)	42. 7	67. 2
Cyanamid (N◇2 per cent ammonium sulphate)	. 05	. 12

FURTHER STUDIES ON THE EFFECT OF CYANAMID AND OTHER NITROGENOUS COMPOUNDS ON BACTERIAL NUMBERS

In continuation of the work discussed above, a much more comprehensive set of experiments was outlined somewhat similar to the first but using several changes in technique suggested by the first experiments. The chief of these was the difference in method of incubation of the soil samples. Instead of using a large sample of soil and taking samples from the same at intervals, a large number of small samples of 50 gm. each were incubated in 250 cc. Erlenmeyer flasks. This eliminated the many objectionable features and sources of error connected with the securing of samples. The mere addition of sterile water to the 50 gm. of soil in the flasks, followed by thorough shaking, made the first dilution.

The soil used was a Clarksville loam which had been in cultivation for several years and was rather low in fertility. To several 50 gm. portions of the air-dried soil in the flasks were added from 5 to 50 mgm. of N in the forms of ammonium sulphate, cyanamid (untreated), cyanamid (oiled and hydrated), dicyanodiamid, guanylurea sulphate, and urea. After thorough mixing, water was added at the rate of 19 cc. per 100 gm. of soil to bring the moisture content up to the optimum. In order that the soils might have better air circulation, the flasks were not plugged with cotton but kept in an incubator containing a large evaporating surface of water which kept the humidity at near saturation. A slow loss of moisture did occur, however, and this was restored at intervals. The experiment, which started on June 18, 1921, was continued at room temperature for various lengths of time, as shown in Table II.

TABLE II.—*Numbers of microorganisms in soil following applications of cyanamid and related compounds*

Treatment (per 50 gm. of soil)	Mgm. N	Millions per gram				
		3 days	7 days	21 days	31 days	38 days
Control		2.04	2.09	1.54	1.26	2.60
Ammonium sulphate	5	1.86	1.54	Lost.	.76	1.99
Do	10	1.90	2.08	.82	1.25	1.77
Do	25	2.18	2.45	.59	1.70	2.95
Do	50	3.91	5.26	1.26	2.52	3.20
Cyanamid, untreated	5	2.58	4.08	.98	1.03	2.72
Do	10	5.52	4.43	1.06	2.23	2.28
Do	25	12.03	8.63	.95	1.81	3.95
Do	50	14.57	6.90	1.22	2.81	2.19
Cyanamid, oiled and hydrated	5	2.14	4.30	1.13	1.24	2.47
Do	10	4.68	2.65	1.41	1.58	3.37
Do	25	13.44	7.95	1.16	1.69	3.72
Do	50	14.53	Lost.	1.30	2.30	3.40
Dicyanodiamid	5	3.23	2.54	2.51	1.55	2.66
Do	10	2.10	2.65	2.22	1.36	1.72
Do	25	2.05	1.21	1.75	1.51	1.86
Do	50	1.65	1.45	1.28	1.32	1.81
Guanylurea sulphate	5	2.00	1.28	1.28	1.40	1.96
Do	10	2.10	1.95	1.03	1.08	1.73
Do	25	2.15	1.43	.89	1.96	2.18
Do	50	2.68	1.50	.93	1.64	2.63
Urea	5	1.97	1.28	.53	1.30	1.58
Do	10	3.05	1.02	.50	1.08	1.70
Do	25	2.76	1.25	.62	1.21	1.72
Do	50	1.99	1.33	.52	Lost.	1.74

The pouring of the plates was done in the same manner as previously, using Lipman and Brown's synthetic agar. The average number of organisms, in terms of millions per gram of soil, is given in Table II for the different treatments. All plates which were badly overrun by fungi or were for other reasons decidedly abnormal were eliminated in determining the averages.

From the data it will be observed that ammonium sulphate produced only a slight increase in the numbers of bacteria and then only with the two largest rates of application. Likewise, dicyanodiamid, guanylurea sulphate, and urea produced no appreciable effects, the slight changes in numbers usually being within the experimental error. On the other hand, cyanamid, whether oiled and hydrated or untreated, produced an increase of approximately 700 per cent at the largest rate of application and smaller increases with lesser amounts. The maximum effect on the numbers of organisms was observed at the first analytical period, namely, 3 days. After 7 days the numbers had decreased to about half and continued to decrease until at the end of 3 weeks there were actually fewer in the treated than in the untreated soils. At the end of the experiment, 38 days, all soils regardless of the kind of fertilizer applied and even the controls showed more microorganisms than at the previous incubation period of 31 days. It is of considerable practical importance to know that the coating of oil which treated cyanamid receives does not appreciably affect the numbers of bacteria in the soil. It should be remembered, however, that most of the organisms which grow on the synthetic agar used are ammonifiers and hence the results are in no way an indication of the effect of the different materials on either the numbers of nitrifiers or the rate of the oxidation produced by them. The conditions required for the optimum growth of the two classes of organisms, especially on laboratory media, are quite different, the nitrifiers being especially susceptible to slight variations in reaction, concentration, source of food supply, and presence of undesirable substances. The ammonifiers are quite hardy and grow under widely different environments.

In comparing the results given in Tables I and II it is necessary to bear in mind the radical differences between the two sets of experiments. The first experiment was with a very fertile soil, the second with a very poor soil deficient in organic matter, nitrogen, and phosphorus. Again, the first work was conducted during the winter months with a soil taken from the field from underneath a crust of frozen soil; the second experiment was made during the hot summer months with soil secured from the field at the time. Furthermore, there were considerable differences, as already noted, in the methods of conducting the experiments, time of incubation, rates of application, etc. All work was done at room temperature which varied during the winter from 15° to 24° C., and during the summer from 21° to 37° C. We could, therefore, scarcely expect closely agreeing results between the two sets of experiments.

The rates at which the changes in the numbers occurred are very different in the two sets of experiments. For instance, in the winter work the maximum numbers were observed in the soils receiving cyanamid at the 31-day incubation period while in the summer studies the maximum numbers were found after 3 days and rapidly dwindled thereafter. There was a marked increase in numbers in both instances, except where too much of the material was used. Of the other materials, ammonium sulphate, urea, and dicyanodiamid produced slightly greater stimulations in numbers in the first set of experiments. A discrepancy occurred in the case of guanylurea sulphate, this material producing a maximum stimulation of 126 per cent with the richer soil and only about 32 per cent with the poorer soil. In fact, in the latter case, the material usually acted as entirely inert, slight depressions in numbers being about as frequent as stimulations.

A STUDY OF THE RELATION OF THE LIME CONTENT OF CYANAMID TO THE INCREASE IN BACTERIAL NUMBERS

Using the same technique as outlined in the preceding experiment, but using the Susquehanna loam soil, another experiment was started on March 27, 1922, in which cyanamid was applied at different rates and the numbers of bacteria developing compared with similar tests where lime was used corresponding to the amount in the cyanamid. The three common forms of lime were used, namely, the oxid, hydroxid, and carbonate, the amounts being calculated on the basis of calcium content. The numbers of colonies which developed on the plates within 4 days are reported in Table III.

TABLE III.—*Numbers of microorganisms in soil following applications of cyanamid and lime*

Treatment (per 50 gm. of soil)	Mgm.	Millions per gram			
		3 days	7 days	14 days	21 days
Control		3.64	3.01	2.17	1.61
Cyanamid	123	12.60	14.00	27.27	2.40
Do	247	8.15	17.60	24.65	7.60
Do	493	3.46	35.26	152.52	.40
Do	123				
$CaCO_3$	570	24.70	45.30	60.20	1.47
Cyanamid	247				
$CaCO_3$	570	6.12	94.53	30.55	1.45
Cyanamid	493				
$CaCO_3$	570	1.68	35.20	69.90	2.60
Do	143	4.35	2.98	1.39	2.12
Do	285	5.82	4.08	2.07	2.13
Do	570	5.57	5.15	1.93	1.30
$Ca(OH)_2$	105	6.05	10.42	2.63	1.17
Do	211	10.25	20.38	14.53	5.13
Do	422	9.95	82.73	30.33	3.67
CaO	80	7.70	8.01	2.38	1.68
Do	160	7.70	20.75	21.58	2.48
Do	319	27.05	92.80	44.40	3.57

It will be noted that cyanamid again produced a very marked increase in bacterial numbers, particularly at the largest rate of application. The time required for the maximum increase was usually 14 days, but this varied slightly with the different treatments. Even at the smallest rate of application of cyanamid, there were about 13 times as many organisms present after 14 days as in the control soil. With the largest application, the increase was 70 times, over 150 millions per gram having been determined by the plate method. Undoubtedly, several millions more were present which did not develop upon the medium used.

The use of calcium carbonate, even though it produced only a comparatively small increase in numbers when used by itself, very markedly increased the numbers when mixed with the two lower percentages of cyanamid. With the largest cyanamid application, the maximum number was 69.9 millions, as compared with 152.5 millions with cyanamid alone. It is difficult to explain this variation, but it was quite likely due to the high alkalinity.

The three forms of lime used produced results about as might have been expected. Calcium carbonate, being neutral and more or less inert, produced only a comparatively small stimulation; the hydroxid, which is basic and quick to act, increased the numbers up to 27 times that of the control within seven days; the oxid, which is still more active, gave a maximum increase of 31 times. If large applications of the oxid are used, as has been done in other experiments

not reported here, the soil is practically sterilized exactly as is the case with heavy applications of cyanamid.

This experiment was planned to determine whether the marked stimulation to bacterial numbers following applications of cyanamid can be largely explained on the basis of its lime content. The results indicate that this is the explanation for a large part of the increase, but evidently the cyanamide and its transformation products as well as the impurities in commercial cyanamid also enter into the results in a very marked manner.

THE RELATIVE IMPORTANCE OF THE CYANAMIDE AND CALCIUM OXID IN CYANAMID IN INCREASING BACTERIAL NUMBERS

In continuation of the work reported in Table III using the same methods and soil, an additional series of experiments was started on June 23, 1922, using pure cyanamide (H_2CN_2) and calcium oxid singly and together in comparison with like applications of cyanamid basing the rates on the nitrogen and calcium contents of the cyanamid. These results are given in Table IV.

Again it will be observed that cyanamid produced a big increase in bacterial numbers. With the three lowest applications, the larger the application the greater the stimulation, but with the heaviest application the numbers were less than with the medium rates, but still far above the control. Using calcium oxid in amounts comparable to that in the cyanamid, large increases in bacterial numbers were also noted and in every case the larger the application the higher the figures. In many respects, the data corresponds to that noted for cyanamid except that the largest application of calcium oxid did not produce a falling off as was observed with cyanamid.

The effect of pure cyanamide was very peculiar. At the two smaller rates, it produced only moderate increases as compared with either cyanamid or calcium oxid, but with 50 mgm. N per 50 gm. soil, 36 times as many bacteria were present at the end of 13 days' incubation as in the control. With double this application, the soil was partially sterilized. It is quite remarkable that cyanamide, at one application produced such a marked effect, while at either slightly higher or lower rates showed only moderate effects.

TABLE IV.—*Numbers of microorganisms in soil receiving cyanamid, pure cyanamide, and calcium oxid*

Treatment (per 50 gm. of soil)	Millions per gram		
	5 days	13 days	21 days
Control	6.26	2.85	1.98
Cyanamid 5 mgm. N	8.03	4.13	2.64
Cyanamid 20 mgm. N	43.96	23.39	9.97
Cyanamid 50 mgm. N	50.80	54.16	5.46
Cyanamid 100 mgm. N	17.20	46.30	14.66
Calcium oxid 16 mgm	11.06	5.30	2.67
Calcium oxid 64 mgm	16.88	8.11	3.50
Calcium oxid 160 mgm	25.90	11.07	7.03
Calcium oxid 319 mgm	71.05	69.26	8.24
Pure cyanamide 5 mgm. N	6.86	3.75	2.16
Pure cyanamide 20 mgm. N	9.81	12.49	6.46
Pure cyanamide 50 mgm. N	9.21	102.84	33.10
Pure cyanamide 100 mgm. N	.71	.34	.46
Pure cyanamide 5 mgm. N+calcium oxid 16 mgm	8.43	6.93	2.87
Pure cyanamide 20 mgm. N+calcium oxid 64 mgm	19.87	25.43	16.16
Pure cyanamide 50 mgm. N+calcium oxid 160 mgm	107.08	52.66	6.76
Pure cyanamide 100 mgm. N+calcium oxid 319 mgm	1.47	5.70	6.14

Where cyanamide and calcium oxid were used together in the approximate proportions found in cyanamid, the figures show clearly the importance of cyanamide in determining the results. At the two smaller rates, there was a fairly marked increase in numbers, probably due more to the calcium oxid than to the cyanamide. With the third highest rate, there was the usual marked stimulation observed with both the lime and cyanamide, used singly, as well as with cyanamid. However, the maximum effects were observed at the 5-day incubation period instead of 13 days as with cyanamide. Evidently, the lime was largely responsible for hastening the bacterial multiplication. With the highest application of the combination of cyanamide and calcium oxide, there was marked retardation at first followed by a small increase over the control later. In this case, the cyanamide was the important factor and neutralized the good effect that the same application of lime produced in the absence of the cyanamide.

EFFECT OF UREA, GUANYLUREA SULPHATE, GUANIDIN NITRATE, AND BIGUANID NITRATE UPON THE NUMBERS OF BACTERIA IN SOIL

After observing the very marked effects of cyanamid on bacterial numbers, it was of interest to extend the previous studies to include some additional transformation products of cyanamide. In this connection, guanidin nitrate and biguanid nitrate were used, as well as guanylurea sulphate and urea, previously studied. All applications were based upon the nitrogen content, the rates being 25–50, and 100 mgm. N per 50 gm. soil. In addition, sodium nitrate was used singly in amounts equivalent to the nitrate contents of guanidin and biguanid nitrates at the highest rates of application. In like manner, a test was included using magnesium sulphate equivalent to the sulphate content of the largest application of guanylurea sulphate. The data secured in this experiment, started on April 20, 1922, using the Susquehanna loam soil, are given in Table V.

In marked contrast to the effect produced by cyanamide, all of the materials used in this experiment produced only slight effects. The increases and decreases noted were usually only comparatively slight and almost within the experimental error. In this particular experiment, a large proportion of these variations may be attributed to the fact that the dilution used in plating was much too small, and consequently a variation of three or four colonies on a plate made a very great difference in the final figures.

TABLE V.—*Numbers of microorganisms in soils receiving nitrogen in the forms of urea, guanylurea, guanidin, and biguanid*

Treatment (per 50 gm. of soil)	Mgm. N	Millions per gram	
		8 days	18 days
Control		1.70	1.20
Urea	25	1.80	1.05
Urea	50	1.77	1.98
Urea	100	2.03	1.15
Guanylurea sulphate	25	1.50	1.98
Guanylurea sulphate	50	1.33	.65
Guanylurea sulphate	100	1.23	.67
Guanidin nitrate	25	1.93	1.15
Guanidin nitrate	50	3.67	.43
Guanidin nitrate	100	1.06	1.67
Biguanid nitrate	25	2.20	1.32
Biguanid nitrate	50	1.23	1.20
Biguanid nitrate	100	3.27	1.25
Sodium nitrate ≎ NO_3 in biguanid nitrate		2.70	1.20
Sodium nitrate ≎ NO_3 in guanidin nitrate		2.23	.83
Magnesium sulphate ≎ SO_4 in guanylurea sulphate		2.30	1.23

SUMMARY

This paper presents the results of laboratory studies on the effect of cyanamide and related compounds on bacterial numbers. In considering these results, it should be borne in mind that the bacterial counts were made on Lipman and Brown's synthetic agar, which, like all media, favors the growth of certain types of organisms; in this instance chiefly the ammonifiers. The use of other substrates would probably have given different results.

Cyanamid produced unusually large increases in the number of bacteria in soils, the maximum increase usually occurring within two weeks after application, but this depended upon a number of factors, chiefly the rate of application and temperature of incubation. In one instance, the number reached was 70 times the control.

In an attempt to determine the particular constituent in cyanamide primarily responsible for this rapid bacterial multiplication, it was found that both the cyanamide content and the lime were very important. Where used alone, either calcium hydroxid or calcium oxid in amounts equivalent to that found in cyanamid produced large increases in numbers. The larger the application, the larger was the stimulation within the limits of these experiments. In the case of pure cyanamide (H_2CN_2) the two lower rates of application produced relatively small increases and the highest partially sterilized the soil. On the other hand, the intermediate rate produced a very large increase comparable to cyanamid. It is, therefore, impossible to state whether lime or cyanamide is the greater factor in explaining the effects of cyanamid. Undoubtedly, both are important in the moderate applications, but the cyanamide or its decomposition products are largely responsible for the partial sterilization with the higher applications. The enormous increases in numbers sometimes noted with certain concentrations of cyanamid can probably be attributed in large part to the cyanamide. Further work is needed to establish these points.

Urea and ammonium sulphate produced only slight effects upon bacterial numbers, even though both materials are known to be excellent sources of plant food for higher plants. As previously pointed out, the absence of effect with urea was most likely due to the fact that the medium used did not contain urea and hence did not favor the urea bacteria. In the case of ammonium sulphate, no marked stimulation could be expected since the nitrogen is already in the ammonia stage and hence not attacked by the ammonifying organisms.

The other materials, namely, dicyanodiamid, guanylurea sulphate, guanidin nitrate, and biguanid nitrate also failed to produce marked effects even though some of them are known to be injurious or at least unavailable for higher plants.

In considering the results presented above, it should be borne in mind that the quantities of materials used were far in excess of the usual field applications. For instance, 50 mgm. of nitrogen per 50 gm. of soil would be the equivalent of slightly more than 10,000 pounds of ammonium sulphate per acre (figured as 2,000,000 pounds of soil). Obviously, no such concentration would ever occur under field conditions except possibly in isolated cases where heavy fertilizations were made in the row. Applications corresponding to field practice, where the material is uniformly mixed with the soil would probably have little effect upon bacterial numbers. Further work is needed along all of the lines treated and broad generalizations are not justified from the data here presented.